IMPERIAL INSTITUTE

OF

AGRICULTURAL RESEARCH, PUSA.

CHEMICAL REVIEWS

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VOLUME IX

PUBLISHED BI-MONTHLY FOR

THE AMERICAN CHEMICAL SOCIETY

BY

THE WILLIAMS & WILKINS COMPANY

Baltimore, U. S. A.

1931

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CHEMICAL REACTIONS AT SURFACES

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The application of the term "catalysis" to a set of chemical phenomena is, in major part, a confession of ignorance. It normally indicates the existence of a series of chemical reactions of which the initial and final states are readily stated, while, of the path between, little or nothing is yet known. When known, the need for the term "catalysis" disappears for the total process becomes a succession of normal chemical reactions. This is true alike of the so-called catalytic reactions in homogeneous systems and of the heterogeneous catalytic reactions, those occurring at surfaces. The use of the term "catalysis" will steadily yield to a more penetrating analysis of the detailed steps of the gross process.

The scientific study of the heterogeneous catalytic reactions has undergone a complete revolution in the last fifteen years, a revolution which justifies the paragraph with which this discussion has been opened. Fifteen years ago these studies were hampered by an assumption that what were in reality fascinatingly interesting chemical processes might be determined in rate by purely physical processes of diffusion. This was an inheritance from some pioneering studies of Noyes and Whitney (1) on the velocity of solution of such substances as lead chloride and benzoic acid in water in which, undoubtedly, the rate of diffusion from a saturated layer next to the solid was the rate-determining factor. These studies were developed with characteristic thoroughness in the laboratories of Nernst (2) and were introduced into the realm of gas reactions by Bodenstein and Fink (3) to explain their beautiful kinetic studies of the velocity of sulfur trioxide forma-

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tion from sulfur dioxide and oxygen at the surface of platinum. The kinetics of this reaction seemed to indicate that the rate of reaction was determined by the rate at which sulfur dioxide could penetrate an adsorbed layer of sulfur trioxide to reach a platinum surface at which oxygen was already present, an instantaneous reaction of the two gases then occurring.

Langmuir initiated the revolution (4) and formulated the plan of campaign. His well-known studies of the reactions of gases with tungsten filaments led inevitably to his concept of surface forces of solids and their saturation by a unimolecular layer of adsorbed gas molecules. The x-ray analysis of crystalline solids by W. H. and W. L. Bragg had revealed both the nature of the unsaturation of the surface atoms and the geometric pattern of the atoms in the plane surface of a solid. The surface of a catalyst, therefore, from Langmuir's point of view, could be regarded "as consisting of a checkerboard in which some of the spaces are vacant while others are filled with atoms or molecules. . . . . When gas molecules condense on the solid surface in such a way that they are held on the surface by primary valence forces involving a rearrangement of their electrons, their chemical properties become completely modified. It is not surprising that in some cases such adsorbed films should be extremely reactive, while in other cases they may be very inert to outside influences. Thus, oxygen adsorbed on platinum reacts readily with hydrogen or carbon monoxide, while oxygen on tungsten, or carbon monoxide on platinum show very little tendency to react with gases brought into contact with their surfaces." Langmuir here stated explicitly what is quite frequently overlooked, namely, that the operation of surface forces might prevent reactions which in the presence of another surface might readily occur. His example of the inability of hydrogen and oxygen to react at a tungsten filament maintained below 1500°K. is, as will be shown later, a very significant illustration of newer ideas concerning surface activity. Langmuir further pointed out that "the specific nature of these various films is quite consistent with the theory that the adsorption depends on typical chemical action. In many cases, especially where we deal with adsorption of large mole-

cules, the orientation of the molecules on the surface is a factor of vital importance in determining the activity of the surface towards reacting gases.

"The reaction which takes place at the surface may occur by interaction between molecules or atoms adsorbed in adjacent spaces on the surface or it may occur between an adsorbed film and the atoms of the underlying solid, or again, it may take place directly as a result of a collision between a gas molecule and an adsorbed molecule or atom on the surface. . . . . When a surface is covered by different kinds of adsorbed molecules distributed at random over the surface, we may expect in general that adsorbed molecules in adjacent spaces should be able to react with one another at a rate which is proportional to the chance that the given molecules shall lie in adjacent spaces."

Thus, in a few swift sentences, Langmuir outlined the direction of progress in the subject, and the research of the past ten years has been abundantly rich in verification of his concepts: elementary space; surface saturation of the solid with unimolecular oriented gas layers, chemically specific in their behavior, reactive and non-reactive; interaction of adjacent molecules or of molecules by collision and at rates determined by the probability of such contact. The program had an insistent appeal, and an intensive development set in at various research centers. Among the more prominent of these may be mentioned the following: Cambridge, England, with Rideal and Constable; Oxford with Hinshelwood; Munich with Schwab; Oppau with Mittasch and Frankenburger; Ludwigshafen with Mark and his collaborators; in this country the Universities of California, Cornell, Johns Hopkins, Virginia and Princeton and also the Fixed Nitrogen Research Laboratory at Washington, D. C. It is with the progress of these studies, their contribution to our present concepts of reactions at surfaces and the new points of view which even the brilliant forecast of Langmuir did not include that the following pages deal.

In the main, two distinct types of research have contributed to the progress achieved. On the one hand, the method employed has been the study of the kinetics of suitable reactions

occurring at sufficiently simple surfaces—a study of the influence of the concentrations of reactants on the velocity with which the reaction proceeded in the reaction zone, the layer of adsorbed gas varying from a sparsely covered surface at sufficiently low reactant concentrations to a saturated unimolecular layer of gas completely covering the surface at sufficiently high concentrations. These studies have been confined practically exclusively to gaseous systems. From the influence of temperature on the velocity and the kinetics of such reactions the energies involved in the reactions have been computed, and comparisons thus facilitated between the reaction energies required when the process occurs at surfaces and when it occurs homogeneously in the gas phase. The other method of investigation has focussed attention not on the reaction but on the surface, its properties and behavior towards potential reactants, studied, in the main, singly. This phase of the work has involved studies of adsorption and heats of adsorption, the influence of extension of the surface and of the enhanced activity of surfaces secured by extension, and the deliberate admixture with the surface material of addition agents which promote or depreciate the surface activity. Conspicuous advances have been achieved also by the skillful combination of both methods of study notably by Pease (5) in the study of the poisoning action of carbon monoxide in the hydrogenation of ethylene on copper, by Russell (6) and Almquist and Black (7) in the study of the action of promotors, and quite recently by Dohse and Kälberer (8) in the elucidation of the mechanism of decomposition of alcohols at the surfaces of typical oxides by studies of reaction velocities in unimolecular adsorbed films.

KINETIC STUDIES OF SURFACE REACTIONS

The adsorption of a gas to form a more or less saturated unimolecular layer of adsorbate is due, according to Langmuir (4), to the time lag between condensation and reëvaporation of the molecules striking the adsorbing surface. Since, on a uniform surface, the rate of condensation r_1 is proportional to the gas pressure, p, the fraction of the surface, $1 - \sigma$, which is bare, and to an accommodation coefficient, α, characteristic of the surface

and expressing the fraction of the collisions which are inelastic (a fraction which is in many cases almost unity) we may write

$$r_1 = k\alpha p (1 - \sigma)$$

or, for a given surface under constant conditions,

$$r_1 = k_1 p (1 - \sigma)$$

Similarly the rate of evaporation, r_2, is proportional to the fraction of the uniform surface covered; hence,

$$r_2 = k_2 \sigma$$

At the steady state of constant adsorption these rates are equal, whence we derive the expression,

$$\sigma = \frac{k_1 p}{k_2 + k_1 p}$$

for the fraction of the surface covered at the pressure p. We may further simplify this to the expression

$$\sigma = \frac{bp}{1 + bp}$$

where $b = k_1/k_2$. From this it is evident that an adsorption isotherm at a given temperature will, in general, contain three readily distinguishable segments. At low pressures and small adsorptions $\sigma = bp$, or, the surface covered varies linearly with the pressure. For high adsorptions where bp is large compared to unity, $\sigma = 1$, and the surface covered is independent of the pressure; it has become saturated. Between these two extremes there falls a curved portion of the isotherm in which the variation with p is intermediate to those just given and which over a sufficiently restricted range of pressures may be expressed adequately by

$$\sigma \propto p^{1/n}$$

where n is greater than unity. A thermodynamic deduction of this adsorption isotherm by Volmer (9) reveals that the satura-

tion point is reached when half the surface atoms are covered with adsorbed molecules. This is attributed by Volmer to a two-dimensional mobility of adsorbed molecules, a phenomenon experimentally established by the work of Volmer and Adhikari (10), who demonstrated such motion of benzophenone molecules on glass and of adsorbed iodine on mercury surfaces.

In a similar manner the fraction of the surface covered by a gas, A, in a mixture of gases A and B, is expressible by the equation

$$\sigma_A = \frac{bp_A}{1 + bp_A + b'p_B}$$

with a corresponding expression for the gas B. A recent experimental study of such adsorption from mixtures by Markham and Benton (11) confirms in part the correctness of such an expression, though in some of the cases studied secondary factors also are operative.

(a) Single reactant

For reactions occurring exclusively at the surfaces in question it is assumed that the reaction velocity obtaining is proportional to the fraction of the surface covered by the adsorbed gas, which, as has been shown, is, in its turn, related to the gas concentration, or pressure p, by the adsorption isotherm. For a single reactant, the three cases already cited of slight adsorption ($\sigma = bp$), complete adsorption ($\sigma = 1$) and partial saturation ($\sigma \propto p^{1/n}$), yield on this assumption three simple characteristic velocity equations. In the first,

$$- dp/dt = k\sigma = kbp = k'p$$

in the second,

$$- dp/dt = k\sigma = k$$

and in the third,

$$- dp/dt = kp^{1/n}$$

These constitute the simplest possible reaction kinetics at surfaces and examples of each have been studied. Each is char-

acteristic.   The first expression is identical in form with that of a unimolecular homogeneous reaction.   The second is unknown in homogeneous kinetics.   The equation is that of a zero order reaction; the rate is independent of the concentration of reacting substance.   The third case, similarly unknown in homogeneous kinetics, is intermediate to the zero and first order reactions. An expression of this form was found by Stock and Bodenstein (12) to represent the decomposition of arsine on glass surfaces covered by arsenic.   The first order rate is given by the decomposition of phosphine on glass (13), of hydrogen selenide on selenium (14), of hydrogen iodide on platinum (15), and by many others.   In contrast to this last case Hinshelwood and Prichard (16) found that the rate of decomposition of hydrogen iodide on gold was independent of the gas pressure and was therefore a zero order reaction.   It is thus evident that the nature of the molecule undergoing change does not itself determine the type of reaction kinetics.   This is determined by the reactant-surface combination and the distribution of the reactant between the surface and the surrounding medium.

With more than one gas involved in the adsorption process at the surface the kinetics become correspondingly more complex. Thus, the unimolecular decomposition of a weakly adsorbed reactant, A, might be modified by the strong adsorption of a reaction product, B.   In such a case, the reaction of A can occur only on the fraction of the surface, $1 - \sigma_B$, which is free of B. Since bp_A in this case is negligible compared with $b'p_B$ we find that

$$\sigma_B = \frac{b'p_B}{1 - b'p_B}$$

and

$$1 - \sigma_B = \frac{1}{b'p_B}$$

Since the reaction rate is proportional to σ_A, and this is in turn proportional to $p_A(1 - \sigma_B)$, it follows that

$$- dp/dt = k \cdot p_A \cdot \frac{1}{b'p_B}$$

This case is of interest for the following reasons.   We may write the equation in the form

$$dx/dt = k \frac{a - x}{x}$$

where a is the initial concentration and x is the amount of product produced in time t.   In its integrated form this equation yields

$$kt = a \ln a - a \ln (a - x) - x$$

For the time of half-change, τ, we derive, by placing $x = a/2$, the expression

$$\tau = \frac{1}{k} (a \ln 2 - a/2)$$

from which it follows that the time of half-life is directly proportional to the initial pressure.   This result is characteristic of zero order reactions.   First order reactions have a half-life which is independent of the initial pressure.   Nevertheless, even though the reaction kinetics yield an equation zero order in nature, the *reaction occurring is unimolecular* and the kinetic equation obtained is determined in form by the retarding effect of the product.   This conclusion indicates most definitely that the order of the reaction is not alone a sufficient guide to the true nature of the process occurring at the surface.   It emphasizes the need for caution in interpreting the results of kinetic studies. A well-investigated case which conforms to this type is the decomposition of isopropyl alcohol on bauxite, studied by Dohse and Kälberer (8).   The reaction rate is retarded by the water vapor produced in the process.   The rate as ordinarily measured is proportional to the concentration of isopropyl alcohol and inversely proportional to the water formed.   By employing a new technique, studying the rate of change of small amounts of the alcohol completely adsorbed by and constituting less than a complete unimolecular layer on the bauxite surface, these authors demonstrated the correctness of this conclusion.

(b) *Two reactants*

Two interacting gases each weakly adsorbed at a surface will occupy fractions of the surface proportional each to its own gas pressure. The chance of reaction will be proportional to the product of these fractions of the surface occupied. The kinetic expression for such a case becomes therefore,

$$- dp/dt = k \cdot p_A \cdot p_B$$

which is the normal bimolecular equation for such a reaction. This behavior is shown, for example, by hydrogen and ethylene on copper surfaces of feeble activity (5, 17). Analogously to the unimolecular case just discussed, such a bimolecular process can be retarded by one of the reaction products, C, the kinetic expression becoming

$$- dp/dt = k \frac{p_A \cdot p_B}{p_C}$$

or,

$$dx/dt = k \frac{(a - x)^2}{x}$$

or, in the integrated form,

$$k = \frac{1}{t} \left(\frac{a}{a - x} - 1 - \ln \frac{a}{a - x} \right)$$

This equation gives the same unimolecular criterion as a simple unimolecular reaction; equal fractions of conversion occur in equal times. The decomposition of nitric oxide on platinum-rhodium surfaces was found by Bachmann and G. B. Taylor (18) to show this behavior, oxygen inhibiting the reaction rate. A simple unimolecular decomposition of nitric oxide would have meant that the surface reaction occurred according to the equation

$$NO = N + O$$

to be followed by rapid combination of nitrogen atoms and of oxygen atoms. The bimolecular reaction inhibited by the prod-

uct oxygen, giving the kinetically unimolecular rate equation, leads to the much more reasonable mechanism for the reaction process

$$NO_{ads.} + NO_{ads.} = N_{2ads.} + O_{2ads.}$$

The retardation is also reasonable since the platinum metals in question are known to have strong affinity for oxygen. This example illustrates well the value of kinetic studies in determining the mechanism of surface reactions, while it emphasizes at the same time the need for care in interpretation of the experimental results.

The kinetic studies cited must serve as representative of a numerous group of similar researches on the reaction kinetics of surface processes. An extended treatment of most of the good data has been given elsewhere (19, 20, 21). It is evident that examples are now known of kinetically simple unimolecular and bimolecular reactions at surfaces; indeed, they are probably more numerous and more reliable than the corresponding gaseous homogeneous reactions. The phenomenon of zero order reactions has been noted and its absence in homogeneous reactions can now be stressed. Third order surface reactions must be rare. The exemplification has been confined to gaseous reactions but the general principles are equally applicable to liquid media (21). In this latter case the conditions are not in general so simple, since the distribution of reactants between surface and medium is not so readily determined. Furthermore, the problem in such systems is complicated by the complexities of solutions in general. There is, however, no reason to anticipate that the mode of treatment found so applicable in gaseous systems cannot be extended in principle to liquid media.

The discussion thus far has been limited to reactions on a uniform surface whereas, as is now well known, the normal reaction surface is a non-uniform structure with varying degrees of surface activity. Certain kinetic studies indicate the necessity of postulating composite surfaces, but there are not many of these. As an example, one might cite the general case of two surfaces on which two reactants, A and B, are respectively moderately

adsorbed. In agreement with the foregoing principles, the rate of reaction would be given by the expression

$$dp/dt = \frac{p_A}{1 + bp_A} \cdot \frac{p_B}{1 + bp_B}$$

where A is adsorbed by one surface and B by the other. Two reactions studied by Hinshelwood and his coworkers obey this expression. They are

$$CO_2 + H_2 = CO + H_2O \text{ on tungsten (22)},$$

$$N_2O + H_2 = N_2 + H_2O \text{ on gold (23)}.$$

For a composite surface containing areas of progressively varying activities it is evident that the total rate would be an integration of a whole series of individual rates, each characteristic of the area on which it occurred. The resolution of the net rate into individual components is probably impossible by simple kinetic studies, though the existence of the multiplicity of rates may thus be indicated. Kinetic investigations at various pressures serve to exhibit the influence of composite surfaces. Thus, according to Schwab and Schmidt (24), the decomposition of ammonia on platinum surfaces in the pressure range up to 0.1 mm. is inhibited by both hydrogen and nitrogen. At higher pressures the influence of nitrogen is negligible. This shows a specific influence of nitrogen on the more active regions of the platinum surface which is not apparent on the more extensive regions of lower activity. Askey (25) found a similar effect of nitrogen in the decomposition of hydrazine on platinum. Donnelly and Hinshelwood (26) have also shown that the kinetics of the hydrogen-oxygen reaction in platinum at atmospheric pressure differ essentially from the results of Langmuir (27) at low pressures, a phenomenon also attributable to variations in the quality of the active surface and to variations in the extent of each type of surface. The most convincing evidence of variable surfaces comes, however, from the study of poisons and promoters of surface reactions and from the correlation of adsorption and activation energy of the reactions with the nature of the surface.

A further discussion will, therefore, be postponed until the results of such studies have been presented.

SURFACE EFFECTS IN CHAIN REACTIONS

The processes thus far discussed occur, under the experimental conditions chosen, only at the surface of the contact agent. The recent discovery of chains of reactions in a homogeneous medium, succeeding an initial process of activation, whether photochemical, radiochemical or even thermal, has opened up the possibility of two new types of surface action. Upon consideration, it may at once be seen that the initiation, by thermal activation, of a chain of reactions might occur at a reaction surface and that, in addition, a chain of reactions might be terminated at such a surface. Both of these possibilities have, within the last few years, been realized experimentally. Actually the termination of reaction chains by surfaces was demonstrated simultaneously and independently by Hinshelwood (28) and by Pease (29). Hinshelwood found that, in a certain temperature and pressure range, the rate of reaction between hydrogen and oxygen was suppressed by packing a silica reaction vessel with coarsely powdered silica. Pease demonstrated the same influence of packing in a variety of oxidation processes of hydrogen and hydrocarbons as well as a somewhat lesser influence of packing in certain processes of polymerization of unsaturated hydrocarbons. The surfaces in question are quite specific in their activity, especially when the chain reactions involve atoms or radicals as the propagating links in the chains. Thus, surfaces of glass coated with potassium chloride reduced the rate of the hydrogen-oxygen reaction approximately one thousandfold as compared with the clean glass surface. While, as will be seen, this effect is due in part to a varying activity of glass and potassium chloride in initiating reaction chains, a like difference in chain-breaking efficiency can be shown.

Hydrogen atoms and chlorine atoms are links in the chain of reactions involved in the hydrogen-chlorine combination:

$$Cl + H_2 = HCl + H; \; H + Cl_2 = HCl + Cl$$

The specific influence of surface in the rate of recombination of halogen atoms is well illustrated by the work of Senftleben and Germer (30). Platinum wire shows a marked tendency to produce recombination of chlorine atoms; the efficiency of the surface in this respect can be materially diminished by glowing the wire in chlorine, a treatment which undoubtedly covers the surface in part with metal chloride. With bromine atoms such treatment was less successful in suppressing recombination. With iodine atoms a further surface factor is of importance. The glass surface of the container causes rapid recombination. Quartz is less efficient than glass in this respect. Polanyi and Bogdandy (31) found that the nature of the surface influenced the length of the chain of reactions in the hydrogen–chlorine combination initiated by the reaction of chlorine with sodium vapor,

$$Na + Cl_2 = NaCl + Cl$$

The chains are short at low working pressures when the glass containing surface is clean but are increased thirtyfold in length after the glass surface becomes coated with sodium chloride. A similar influence of walls on atom recombination is manifest in the chain reaction involved in the photochemical formation of phosgene and in the photosensitized oxidation of carbon monoxide in the presence of chlorine (32).

The effect of surfaces on the recombination of hydrogen atoms has been known since Wood's initial work (33) on the production of atomic hydrogen in discharge tubes. Water vapor adsorbed on the containing glass walls increases the yield of atoms as it poisons the glass walls, reducing their surface activity for recombination. H. von Wartenburg and Schultze (34) have shown that hygroscopic materials, notably sirupy phosphoric acid, are similarly efficient. Bonhoeffer (35) showed that the order of efficiency of metals in hydrogen atom recombination was that of their activity as hydrogenation catalysts. Taylor and Lavin (36) extended these observations to the recombination of hydrogen atoms and hydroxyl radicals. It was in the course of this work that it was shown that potassium chloride coated surfaces were superior to clean glass surfaces in promoting the recombina-

tion of hydrogen and hydroxyl.   It was also shown that dehydration catalysts are very efficient for this latter recombination but are of negligible efficiency in hydrogen atom recombination. The specificity of surfaces for both molecular and atomic reactions is thus patently demonstrated, and the correlation of the activity of surfaces in atomic reactions with similar processes involving molecules leads to a considerable simplification of the problem involved in such cases of specific action.   Once it is ascertained what factors determine the rate of recombination of hydrogen and hydroxyl at a surface, the same factors will be involved in the hydration of, and elimination of water from, molecular species. Similar considerations concerning atomic hydrogen recombination will be involved in surface reactions of hydrogenation and dehydrogenation.

The demonstration that reactions at surfaces may give rise to chemical reactions in the gas phase surrounding the catalyst has recently been supplied by the researches of Alyea and Haber (37) with hydrogen-oxygen mixtures and by Thompson (38) with carbon disulfide-oxygen mixtures.   These authors, in studying the mechanism of these two chain reactions, found that no reaction occurred when two independently heated streams of the gases in question, e.g., hydrogen and oxygen, were allowed to impinge on each other away from surfaces.   The introduction of certain surfaces into the crossed streams at their junction brought about the same rapid reaction that would have occurred if the two gases had been heated together to the given temperature in a single tube.   That the effect of the surface was specific and not due to the additional mixing brought about by the introduction of the surface is evident from their observations that while glass, quartz, platinum, iron and copper surfaces produced the rapid change, an aluminum surface was without effect.

The initiation of chain reactions in hydrogen-oxygen systems by atomic hydrogen produced in the photochemical decomposition of ammonia has recently been demonstrated by Farkas, Haber and Harteck (39).   They have shown that the chains so initated increase in average length with temperature, the chain length being 25 at 290°, 380 at 405° and many thousands at 500°C.

The efficiency of atomic hydrogen in thus initiating chains in the hydrogen-oxygen reaction at once suggests that the chains initiated at surfaces might extend into the gas phase by liberation into the gas of atomic hydrogen as a result of such surface reactions. The evidence for such an interpretation has recently been collected by Alyea (40) and this evidence is supported by earlier data already in the literature.

Several years ago a number of publications (41, 42) dealt with the production of "active" hydrogen by various methods of activation, included in which were methods involving the use of catalytic agents, notably platinum. In many of these cases claims for the production of active hydrogen were made under circumstances which seemed to violate the laws of thermodynamics. In other cases, it was experimentally demonstrated that the production of hydrogen in an active form was associated with the occurrence of some chemical reaction. In one case in particular, the formation of active hydrogen was associated with the presence of small amounts of oxygen in the hydrogen gas which was led over a platinum catalyst, the exit gas from which was shown definitely to contain an active form of hydrogen over a stretch of 5 cm. from the catalyst material (43). In these experiments adequate experimental controls of the activity were employed. Since the reaction was carried out at low temperature (<120°C.), effects due to electron emission and ionization should have been entirely absent. Mitchell and Marshall finally concluded that the activity was due to the production of small amounts of triatomic hydrogen. It is now apparent that the active hydrogen was in reality atomic hydrogen and that the method employed by Mitchell and Marshall is a general method applicable at a wide variety of surfaces.

The paper by Mitchell and Marshall provides the most conclusive evidence of the liberation of atomic hydrogen into the gas stream. Low temperature activation of pure hydrogen by contact with platinized asbestos was shown not to occur. Small concentrations of oxygen, down to 0.02 per cent, were shown to promote the activation and there was combustion of hydrogen during the process. The active hydrogen produced under these

conditions was unstable and persisted for a distance of 5 cm.
from the activating surface under the experimental conditions.
The activity varied slightly with the time of contact. The
active hydrogen reduced copper oxide at 82°C., whereas the
unactivated gas did not reduce the same samples of copper oxide
below 116°C. It was definitely shown that the difference in
reduction temperatures was not due to the formation of copper
nuclei in the oxide at which reduction might then occur at the
lower temperature without platinum adjacent (44). There was
also no effect due to water vapor produced on the platinized
asbestos, for it was found that most of the water formed was
retained on the platinized surface for considerable periods of
time. In the earlier experiments of Anderson (45), which pre-
ceded this study by Mitchell and Marshall, active hydrogen from
both platinum and palladium was also shown to react with sulfur,
yielding hydrogen sulfide under conditions which would not yield
this product with normal molecular hydrogen. Mitchell and
Marshall also record the significant experiment performed by
Dr. S. Judd Lewis, at the suggestion of Mr. C. Campbell, which
demonstrated that the active hydrogen produced in this manner
reduced the unsaturated groups in olive oil.

Recent experimental work, as well as theoretical evidence
based on the Pauli exclusion principal and the new quantum
mechanics, point definitely to the nonexistence of triatomic
hydrogen. The reactions of the active hydrogen just recorded
are all possible reactions of atomic hydrogen as demonstrated
by a voluminous literature (46) in recent years. The absence of
any effects when the hydrogen is pure shows definitely that the
activity is not due to any such small concentration of atomic
hydrogen as would be in equilibrium with molecular hydrogen
after passing over a catalyst at the given temperature. The
equilibrium concentration in the system $H_2 = 2H$ at the tempera-
ture in question represents the maximum amount thus obtainable,
and this is vanishingly small. It is necessary then to formulate
the chemical reactions of hydrogen with oxygen which at sur-
faces will yield atomic hydrogen. On platinum and palladium
it is known that oxygen is strongly and irreversibly adsorbed.

If we assume that it is present on such a surface as atomic oxygen strongly bound to the surface there are immediately suggested two possible reactions capable of yielding hydrogen atoms to the gas phase. If we represent the oxygen covered surface by $S(O)$, the two possible reactions are

$$S(O) + H_2 = S(OH) + H \uparrow \qquad (1)$$

and

$$S(OH) + H_2 = S(OH_2) + H \uparrow \qquad (2)$$

Disregarding the heats of adsorption of the several species on the surface, we know, from the new experimental data on the heats of formation of hydrogen and water from the atoms (47), that both of these reactions are exothermic in the gas phase and thus provide a possible mechanism for the release of atomic hydrogen into the gas stream. It is evident that, if both of these reactions are possible, since O_2 will yield 4H, atomic hydrogen up to four times the oxygen concentration of the gas might be thus produced. This would represent the maximum attainable, and, in practice, concentrations well below this could be anticipated, since there is always the possibility of surface reactions of adjacently adsorbed oxygen and hydrogen atoms,

$$O_a + H_a = OH_a$$

and of adjacently adsorbed hydroxyl and hydrogen atoms

$$OH_a + H_a = OH_{2a}$$

For a surface initially saturated with hydrogen, the amount of atoms that could be liberated would be smaller. The reaction

$$H + O_2 = OH + O$$

is, on the best available evidence, somewhat endothermic. The collision of an oxygen molecule with two adsorbed hydrogen atoms could however yield two adsorbed hydroxyls, since the reaction

$$O_2 + 2H = 2OH$$

is strongly exothermic. The reaction of such adsorbed hydroxyls with molecular hydrogen to yield adsorbed water and atomic hydrogen according to reaction 2 above would thus again produce, in the gas phase, atomic hydrogen, but to only half the extent of that possible with a surface initially laden with adsorbed oxygen.

The mechanisms just cited provide an explanation not only of the active hydrogen noted by Mitchell and Marshall but also of the effect of surfaces in initiating chains of reaction in hydrogen-oxygen mixtures. It is known that atomic hydrogen produced in hydrogen-oxygen mixtures at low temperatures leads to the formation of hydrogen peroxide, often to the exclusion of water formation. It is of interest to recall in connection with the suggestions just put forward for the initiation of chains in such mixtures at higher temperatures that Pease (48) has recently demonstrated the formation of peroxide in the thermal reaction of hydrogen and oxygen under conditions which involve the chain mechanism. The reactions of such mixtures with atomic hydrogen at low temperature are thus adequately correlated with the data on reaction chains. The varying efficiencies of different surfaces noted by Alyea and Haber (37) are also in accord with the point of view here put forward. At surfaces of glass, quartz, platinum, iron and copper, the necessary activation of either hydrogen or oxygen can be secured at the operating temperatures used (49). The case of aluminium is the illuminating exception. The metal surface was undoubtedly covered with a thin film of adherent oxide. All the experimental evidence indicates that on such alumina surfaces neither hydrogen nor oxygen show any evidence of activation. As Taylor and Lavin (36) showed, alumina was quite inert in bringing about the recombination of hydrogen atoms and hence must be equally inert in activating hydrogen molecules. The absence of any evidence on the effectiveness of alumina as an oxidation catalyst is evidence against oxygen activation on this material. An oxide-coated tungsten filament constitutes a case parallel to that here presented by oxidized aluminium surfaces. As is well known from the investigations of Langmuir, neither activation of hydrogen (formation of atomic hydrogen) nor oxidation of

hydrogen (formation of water) occurs on such surfaces below 1500°K. It is very evident from experimental data, therefore, that on these surfaces there is no mechanism possible by which atomic hydrogen can escape from the surface into the gas phase; otherwise the chain reaction obtaining when other surfaces are present would also occur with the oxide-coated tungsten surface.

These considerations lend interest to the recent paper by Bennewitz and Neumann (50). By the use of a novel method dependent on the torque of a platinum foil only one half of which was activated, these authors were led to the conclusion that the major portion (> 99.9 per cent) of the hydrogenation of ethylene must occur as a chain reaction in the gas phase. This conclusion is not yet in accord with the results of work with atomic hydrogen produced by excited mercury atoms. Here all the most recent data (51, 52) indicate that the chains are nonexistent at room temperatures. There is, however, the possibility that, at higher temperatures, the reaction may take on the chain characteristics. By analogy with the preceding case of oxygen one might suggest the following sequence

$$H_a + C_2H_4 = C_2H_{5(a)} \text{ (Surface reaction)} \tag{1}$$

$$C_2H_{5(a)} + H_2 = C_2H_{6(a)} + H \uparrow \tag{2}$$

the atomic hydrogen produced in reaction 2 being released into the gas phase. The most recent data by Mecke (47) on the energy of binding of C_2H_5 and H make this reaction a definite possibility, although we have elsewhere (53) cited evidence against its frequent occurrence at room temperatures. To test this matter further, we are at present making experiments on the temperature coefficient of the hydrogen-ethylene reaction under the influence of excited mercury.

Concerning the mechanism of initiation of the carbon disulfide-oxygen chain at glass surfaces studied by Thompson (38), since the reaction is initiated by glass at as low as 140°C. and since glass shows no capacity to activate oxygen at such temperatures, one is led to the conclusion that the surface must activate the disulfide. The chain-initiating reaction on this basis

may involve the projection of an oxygen atom into the gas phase
by some reaction of which

$$S_{(a)} + O_2 = SO_{(a)} + O \uparrow$$

is one possible example.   It is now experimentally established
that oxygen atoms react quantitatively with carbon disulfide
(54) to yield various oxidation products.   That the presence of
oxygen atoms in such carbon disulfide-oxygen mixtures may lead
to a reaction chain is evident from some unpublished data ob-
tained by Emeleus in this laboratory.   Emeleus has shown that
the ignition temperature of such mixtures is quite definitely
lowered by introduction of ozonized oxygen into the system.
With 0.5 per cent ozone an especially violent explosion was
obtained with the gas mixture at as low a temperature as 55°C.,
markedly inferior to that noted above in the thermal reaction in
glass vessels.

   In the case of saturated hydrocarbon oxidation, there is definite
evidence also that the chains of reaction originate at the surface
of the containing vessel as well as terminate at the same.   Thus,
Pease (55) found that packing very considerably reduced the
rate of oxidation of propane and the butanes, pointing to inhib-
itory action.   However, in a packed tube coated with potassium
chloride, the rate of reaction was materially less than in the
packed tube without the coating.   This definitely indicates a
positive effect of the glass on the initiation of chains.   In a large
empty tube coating had little or no effect.   This might mean one
of two things; either that the reaction was almost exclusively
homogeneous, or that chains once initiated at the walls were
extremely long.   The evidence from the packed vessels supports
the latter alternative.   Bone and Hill (56) in a recent paper on
the oxidation of ethane at 300°C. report other data in accord with
the concept of chains initiating at the wall.   The slow combustion
of this gas shows a definite and sometimes prolonged induction
period which can, however, be eliminated by introducing a variety
of substances, for example, acetaldehyde, into the reaction mix-
ture.   There is reason to believe that during the induction period
a slow surface reaction occurs which liberates a chain initiator

into the gas phase. By analogy with the hydrogen-oxygen case, this reaction might be between oxygen molecules and the methyl radicals formed at the surface by adsorption of ethane; thus,

$$CH_{3ads.} + O_2 = CH_2O + OH \uparrow$$

the OH being liberated into the gas phase. This reaction of gaseous methyl has now been well established by the work of Bates and Spence (57). The gradual evaporation of the formaldehyde thus formed would lead to autoacceleration of reaction, since Bone and Hill showed that such was the effect of added formaldehyde. The OH would have a certain capacity as chain producer by reason of the reactions,

$$C_2H_6 + OH = C_2H_5 + H_2O$$

and

$$C_2H_6 + OH = C_2H_5OH + H$$

The ethane molecule might also be adsorbed on the surface as $C_2H_5 \cdot H$, in which case acetaldehyde would be formed on oxidation and made available by evaporation to the gas phase.

It was also shown that there was a pronounced effect of drying on the rate of ethane oxidation. This again points to the intervention of surface in the reaction, since it is difficult to account for an effect of minute traces of water vapor on a homogeneous gas reaction. The water effect may, however, mean an increased efficiency of the surface, when dry, in ending reaction chains.

There is no such definite evidence of surface efficiency in initiating reaction chains in the oxidation of the unsaturated hydrocarbons, ethylene and acetylene (58), though, in these cases, definite experimental test of such a possibility is lacking and would constitute a material contribution to our knowledge of mechanism in these reactions.

THE CHEMICAL COMPOSITION OF ACTIVE SURFACES

An accumulation of data relative to reactions at surfaces has revealed that certain types of elements and compounds are always associated with certain types of surface reaction. It is this speci-

ficity of surface chemical composition for particular reactions which most directly suggests the chemical nature of the surface processes preliminary to the actual reactions involved. Lacking the scientific explanation of this association of surface with reaction, the experimentalist exercises his choice of surface rather as one empirically learned in the art than as a scientist.

Reactions of hydrogenation or reduction as well as of dehydrogenation are most generally effected on one or another of a restricted series of metals or, as a result of recent researches in this field, on a similarly restricted and well-defined series of oxides or mixtures of the same. The principal metals are the platinum metals, nickel, cobalt, iron, and copper, with a few others of much less importance, such as silver, cadmium, and tin. The oxides include those of zinc, manganese, and magnesium, generally with chromium oxide in admixture as a promoter. Many more oxides have, in addition to hydrogenation-dehydrogenation activity, a marked and simultaneously manifested efficiency as dehydration catalyst; among them may be mentioned the oxides of the rare earths, cerium, praseodymium, neodymium, samarium, gadolinium, dysprosium, and oxides of earths of the ytterbium series as well as of indium, scandium, yttrium and lanthanum (59). Some recent evidence indicates that certain sulfides, notably molybdenum, cobalt, nickel, chromium and iron sulfides, may also function as hydrogenating surfaces, especially for reaction with sulfur compounds (60).

For hydration and dehydration processes a series of oxides which include alumina, silica, thoria, zirconia and tungsten oxide are especially to be recommended for their freedom from simultaneous dehydrogenation activity. The efficiency of certain of these oxides is improved by conversion to the sulfate or phosphate. Furthermore, the phosphates and pyrophosphates of oxides with pronounced dehydrogenation activity as, for example, zinc and manganese oxides, are mainly dehydrating in activity.

Of the metals at which oxidation processes are effected, platinum is the most important technically as well as in fundamental research. Rhodium, palladium, and osmium are also important members of this group. Silver and gold are used for the same

purpose. Oxides are the most important class of compounds whose surfaces accelerate oxidation processes. The most important members of this class of contact agents are oxides having several stages of oxidation. Oxides of vanadium, iron, manganese, molybdenum, tungsten, uranium, lead, copper, silver, cobalt, and nickel may be instanced. For certain purposes salts with such oxides as acidic constituents are convenient as, for example, the variadates. The well-known case of Hopcalite illustrates the increased efficiency of mixed oxides over the constituents used singly.

The introduction of and removal of halogens from compounds may be effected at surfaces of carbon, sulfur, iodine, iodine monochloride and the halides of elements having polyvalence. Aluminium, vanadium, molybdenum, bismuth, zinc, tin, copper, and iron chlorides may be instanced. This type of catalyst may also be used for reactions involving addition or removal of the hydrogen halides.

It is a problem of the future to elucidate the reasons underlying this definite association of surface type with reaction type. A start has been made in the solution of this problem by recent studies, subsequently to be discussed, of the hydrogenation-dehydrogenation contact agents. It remains to be seen how the conclusions from such work can be generalized. Progress in this direction can best be indicated by reference to the work carried out on the physical and chemical properties of such contact surfaces.

THE PHYSICAL-CHEMICAL PROPERTIES OF ACTIVE SURFACES

It is obvious that, for reactions at surfaces, an extension of surface as great as practicable within the limits of the experimental conditions is desirable. Extension of surface alone is not, however, the sole criterion of successful surface action. The researches of the last decade have abundantly demonstrated, in a variety of reactions and at various surfaces, that, in addition to a quantitative extension of surface, the quality of that extended surface is of primary importance. The technique of the worker in this field is directed towards securing simultaneously with

extension of surface a high specific reactivity. The methods to be employed in the characterization of a reaction surface are, therefore, of manifest importance in this problem of surface action. It is apparent that, by processes of trial and error, suitable methods for achieving the desired reaction may be obtained. These will in general, nevertheless, be but empirical efforts, without a truly scientific basis of operation. The scientific development of method in surface chemistry must come from a deeper appreciation of the factors operative in the characterization of the surface. This really reduces the method to a study of the physical-chemical properties of surface materials upon which particular chemical processes may be achieved. The realization that adsorption at a surface is a condition precedent to reaction at such surface and that the phenomenon of adsorption becomes the more readily manifest with large extension of surface naturally provided an impetus to the correlation of adsorption and reaction at the adsorbing surfaces. A peculiarly fruitful field of study was thereby discovered. We may illustrate this development by reference to surfaces promoting hydrogenation-dehydrogenation reactions since, with these, the correlation is more complete than with any other of the typical processes and surfaces. The application of the methods of study used with hydrogen to other reacting gases would constitute an invaluable additional contribution to our knowledge of surface reactions.

An examination of the adsorptive capacity for hydrogen of a number of typical metal surfaces such as those of nickel, cobalt, iron, copper, platinum, and palladium (61) showed them to possess high specific adsorptions for hydrogen. Low adsorptive capacity was associated with low surface reactivity (62). It was shown that the extent of adsorption per unit weight of metal was determined by the method of preparation, by distribution on inert supports, by subsequent treatment of the surface with poisons, or by heating (63). In many respects, however, the adsorption was different from the adsorption of gases or vapors by inert adsorbents such as silica gel or carbon. The adsorption of hydrogen on such surfaces was definitely specific, in no way akin to a non-specific, physical condensation on inert surfaces.

The adsorption was accompanied by high heat effects, heats of adsorption of the order of 10,000 to 30,000 calories per gram-molecule of adsorbed hydrogen being common (64, 65, 66, 67, 68, 69). These contrast with the heats of adsorption of the order of magnitude of the heat of liquefaction, 450 calories in the case of hydrogen, usual with non-specific adsorbents. The adsorption isotherms were characterized (63) by saturation at low partial pressures (of the order of 100 mm. or less) of the gas, in contrast with the increased adsorptions at even high pressures with non-specific adsorptions. Of importance in the subsequent development was the observation that these apparent saturation values for hydrogen adsorption on metal surfaces decreased with increasing temperature, calculation indicating that a gas pressure of many thousands of atmospheres would be required to increase the adsorption by the small amount which differentiated the apparent saturation values at the two given temperatures.

The necessity for drawing a distinction between such different types of adsorption has become increasingly evident with progress in the study of adsorption at active surfaces. It has become customary to speak of "primary" and of "secondary" adsorptions; in other cases there has been a differentiation attempted between "reversible" and "irreversible" adsorptions; "physical" and "chemical" adsorptions have been suggested. No precise method of identification of one or the other type has been available nor has it been possible to state whether one or both types were present in a given case. In the theoretical treatment of adsorption there has been a tendency to discuss only the non-specific type and to eliminate from consideration the chemical type of adsorption. Quite recently, definite progress in such differentiation has been achieved. The study by London (70) of the nature of molecular forces from the standpoint of modern wave mechanics has recently led to a quantitative formulation of adsorption on the assumption that the gas in the adsorbed layer has the same equation of state, i.e., van der Waals' forces, as in the gas phase and that the forces of adsorption and van der Waals' forces are related. The equation obtained yielded satisfactory data for the heats of adsorption of helium, nitrogen, argon, carbon

monoxide, carbon dioxide and methane on charcoal. It is, therefore, evident that in these cases of non-specific adsorption the adsorbed gas is molecular in nature and the adsorption forces are van der Waals' molecular forces which are sharply distinguishable wave-mechanically from electrostatic and valence forces.

In the second place, it has recently been found by experimental measurements (71, 72, 73, 74) that certain gases can be adsorbed on particular surfaces in at least two different ways characterized by the extent of adsorption and its variation with temperature. The experimental evidence indicates that the low temperature adsorption is non-specific, molecular, and rapid, condensation probably occurring from a large fraction of the collisions with the adsorbing surface. On the other hand, a rapidly accumulating set of data indicate that there supervenes in a higher temperature range a slow, specific adsorption which may indeed be considerably more pronounced in the amount of gas adsorbed than the low temperature adsorption. The velocity with which this high temperature type of adsorption occurs increases exponentially with the temperature so that one can speak of the activation energy of the adsorption process in the same manner that one speaks of the activation energy of chemical reactions. The magnitude of these activation energies of adsorption varies from case to case and depends on the nature of the gas adsorbed, on the chemical composition of the adsorbent and also on the qualitative nature of the surface area (74).

The detailed theoretical analysis of the concept of adsorption with accompanying activation energy has recently been given elsewhere (73). It will suffice here to indicate some of the consequences of the concept in so far as they relate to the problem of surface reaction, and to cite some of the more conspicuous experimental data in verification of the conclusions reached. Adsorptions having activation energies of a sufficient magnitude will not be experimentally realizable at low temperatures due to inadequacy of activation energy at the temperature in question. If, as seems evident, the van der Waals' molecular adsorptions occur with little or no activation energy, this type of adsorption will occur practically exclusively in the lower temperature ranges.

At high temperatures, the adsorption with activation energies will predominate, and, if the heat of adsorption of the activated adsorption is higher than that of the van der Waals' adsorption, the extent of adsorption will be greater in the higher temperature range than in the lower. This phenomenon, which is quite contrary to any unmodified adsorption theory, will be shown to yield an important experimental criterion of activated adsorption. A second important consequence of the concept of adsorption with accompanying activation energy will also be useful. The low values of activation energy for van der Waals' adsorption imply that the velocity of adsorption will be extremely rapid. This idea concerning velocity of adsorption has been consistently held. On the other hand, with activated adsorptions having high activation energies, there will be definite ranges of temperature in which the adsorption occurs at rates either too slow or too fast to measure, but there will also be an intermediate range of temperature in which the velocity will be at a tempo convenient for experimental measurement. From such measurements, as in the kinetics of chemical change, the variation in velocity with temperature will permit a calculation of the activation energy of the adsorption process.

The best data already available in the literature indicative of increased adsorption at higher temperatures are those of Benton and White (71) for the adsorption of hydrogen on nickel, extending the earlier studies of Gauger and Taylor (63). These experiments, when combined, provide comparative data for adsorption on nickel extending from $-209°$ to $305°C$. The data of Benton and White exhibit a minimum at about $-190°C$. Between $-190°$ and $-110°C$. there is approximately a fourfold increase in adsorption. Above this temperature range, at various pressures, there is again a decreasing adsorption with increase of temperature, the decrease being the more pronounced the lower the operating pressure. At an operating pressure of 60 cm., in the temperature range $-110°$ to $0°C$., the adsorption is constant. The surface is apparently saturated with the activated adsorbate. Above $0°C$., at the same pressure there is a normally decreasing adsorption with increase in temperature. The fact

that the increase in adsorption with temperature occurs in the low temperature range of $-190°$ to $-110°C$. indicates that the activation energy of the activated adsorption of hydrogen on nickel cannot be very large. We shall discuss in subsequent paragraphs cases where the increase of adsorption occurs in higher temperature ranges and for which we have accumulated measurements of velocity at various temperatures, and hence deduced the activation energy. We can, however, note even from Benton and White's qualitative observations that the velocity of adsorptions measured by them are in accord with the concept of our assumed van der Waals' adsorption, "the pressure became constant almost immediately after admitting the gas." In the higher temperature range, equilibrium was fairly rapidly established at very low pressures, which agrees with a rapid velocity of activated adsorption on the most active portions of the surface where the activation energy is lowest. At moderate pressures, the velocity was slower, corresponding to the somewhat higher activation energy of the intermediately active areas of the surface, while, at higher pressures, when, it is obvious, the most active areas are mostly covered, the velocity of adsorption was the slowest. The existence of a slow velocity of activated adsorption is consistent with the observation of Gauger and Taylor (63) that the desorption curve of nickel always showed larger amounts of adsorbed gas at a given temperature and pressure than those obtained in the adsorption experiments. As has been pointed out elsewhere (73), the existence of high heats of adsorption and of maxima in the heat of adsorption versus amount adsorbed curves are also in accord with the concept of slow activated adsorption in the higher temperature range ($-110°$ to $305°C$.).

Much more decisive experimental data demonstrative of the existence of two types of adsorption of hydrogen, one with low activation energy, the other with higher activation energies, have been accumulated in recent months by Mr. A. T. Williamson and the writer in Princeton. As has been pointed out in a preceding section, as a result of recent industrial developments a restricted series of oxides and mixtures of oxides now finds extended use as hydrogenation agents. These oxides are operative for certain

hydrogenation processes in a higher temperature range than the corresponding metals. These offered the possibility that they would show, in a higher temperature range, the same adsorption phenomena as noted by Benton and White for hydrogen on nickel in the region below room temperatures. Furthermore, by measurements of adsorption velocity and its variation with temperature, the actual magnitudes of the activation energies could be ascertained in place of the qualitative observations with nickel cited above. The results obtained are abundantly confirmatory of the views under consideration. We have operated with a surface of manganous oxide and, for a surface of higher specific activity, with a mixture of manganous and chromium oxides. The experimental results will be detailed elsewhere. Here, only a summary need be presented. At $-78°$C., the adsorption of hydrogen on a given sample of manganous-chromium oxide is small, rapidly attained and mostly reversible by evacuation at the same temperature. We assume this to be adsorption of the van der Waals' type. At $0°$C., the adsorption of hydrogen in this manner is less than at $-78°$C., but at this temperature there sets in an extremely slow adsorption, the rate of which makes perceptible changes in adsorption measurable only over periods of hours and days. At $100°$C., and at $132°$C., where the van der Waals' adsorption has fallen to negligible proportions, the rate of this activated adsorption has now risen to conveniently measurable velocities, and, from such measurements, activation energies have been calculated. The velocity of this activated adsorption increases rapidly with temperature as measured at $184°$ and $305°$C., but, and this is more striking, the amount of adsorbed hydrogen has *risen to fifteenfold that obtaining under similar pressure conditions at $-78°$C.* At $440°$C. the extent of adsorption is still severalfold that at $-78°$C., but definitely less than at $305°$C. under the same pressure conditions. The increased adsorption is a real adsorption phenomenon, completely reversible, all of the hydrogen being recoverable by evacuation, though requiring continued pumping for long intervals of time at temperatures around $460°$C. We have no evidence of water formation with these adsorptions, presumably because of the well-known irreducibility of manganous oxide.

From the velocities of adsorption at 100° and 132°C., the activation energy of the adsorption process may be calculated by means of the equation

$$d \ln v/dT = E/RT^2$$

In this way we have found that, on the most active portion of the surface (10 cc. adsorbed) the velocity is relatively rapid and the activation energy small (E = 5900 calories). With increasing surface covered, the velocity of adsorption sinks, whereas the activation energy rises consistently. The mean activation energy on the surface covered by the first 25 cc. of adsorbed gas amounts to 8500 calories. These data add another factor to those important in the heterogeneity of reaction surfaces. It is evident that, on the active centers, the velocity of adsorption is very much more rapid than on the less active areas of the surface. Thus, for example, on two areas of the surface at present under discussion having, let us assume, activation energies of 3000 and 5000 calories respectively (areas covered in the adsorption of the first 10 cc.) the velocities of adsorption at a temperature of 127°C. (= 400°K.) are in the ratio $e^{-3000/2.400} : e^{-5000/2.400} = e^{2000/800} : 1 = e^{2.5} : 1 = 12.18 : 1$. The velocity is twelvefold more rapid on the former than on the latter.

Furthermore, it is well known that the efficiency of surfaces for reactions can be enormously enhanced by the use of promotor agents, and that the promoter may produce not only an extension of the surface but also a qualitative improvement of the surface. That the promoter may also yield a material change in the velocity of adsorption is evident from a comparison of the data cited above for manganous oxide promoted with chromium oxide and data obtained with an unpromoted, though fairly active, sample of manganous oxide prepared by controlled ignition of the oxalate. With this latter preparation, while the general behavior as to adsorption was the same as with manganous-chromium oxide, temperatures at which comparable velocities of adsorption were obtained were more than one hundred degrees higher. Also, from the velocity data at 184° and 305°C. it was found that the activation energy of hydrogen adsorption on the most active

centers of the manganous oxide surface was about 10,000 calories. This means that the ratio of adsorption velocities on the promoted and unpromoted surfaces is given by the expression $e^{-(10,000-3,000)/RT}$ $= e^{+7,000/RT}$ or, for a temperature of 227°C. (= 500°K.), a value $e^{7,000/1,000} = e^7$ or approximately 1,100. It is very evident, therefore, that, for any reaction in which velocity of adsorption or desorption is the rate-determining factor, the employment of a promoted surface would be of enormous assistance. It is probable that this factor of velocity of activated adsorption is the one in which the function of promoters is most significant in a large number of reactions, since evidence is accumulating that what we have here demonstrated for hydrogen adsorption can be generalized for many gaseous reactants.

One additional and most definite piece of evidence as to the fundamental distinction between the rapid low temperature adsorption of hydrogen on manganous-chromium oxide surfaces and the slower but more pronounced adsorption of hydrogen in the higher temperature range is forthcoming from the heats of adsorption of the gas on the surface in the two temperature regions. As is well known, the heat of adsorption can be calculated from any two isotherms on which the equilibrium pressures for a given amount of adsorbed gas have been determined. The equation connecting heat of adsorption, pressure and temperature is

$$\log p_1 - \log p_2 = \frac{\lambda}{4.58}\left(\frac{1}{T_2} - \frac{1}{T_1}\right)$$

where λ is the heat of adsorption, p_1 and p_2 are the equilibrium pressures for a given adsorption at T_1 and T_2, respectively. Using this equation Williamson's data show that in the temperature region $-78°$ to $0°C.$, λ has a value of about 1,900 calories per mole, whereas, from the isotherms at 305° and 444°C., a heat of adsorption greater than 19,000 calories per mole is calculated. The distinction between the calorimetric effects of the two types of adsorption is manifest. The magnitude of the divergence serves also to account for the sharp increase in amount adsorbed in the two ranges of temperature.

The spin isomerization of hydrogen

There exists, fortunately, in the ortho-, para-hydrogen interconversion a surface reaction which involves only one molecular reactant and product, hydrogen. This process, which may be characterized as one of spin isomerization, undoubtedly involves some form of activation of the hydrogen molecule in the association with the surface which produces one or another form. For there is evidence in the data of Bonhoeffer and Harteck (75) dealing with this subject that the influence of surface is quite specific and that adsorption alone is not sufficient to bring about the isomerization. It is for this reason that the writer has already suggested elsewhere that, on the surface of charcoal, even at liquid hydrogen temperatures, the adsorbed hydrogen must be in part in the activated form. This spin isomerization may, therefore, serve as a convenient reaction process for an auxiliary test of the ideas concerning the nature of hydrogen adsorption discussed in the preceding paragraphs.

Bonhoeffer and Harteck found nickel to possess a negligible activity for the reconversion of para-hydrogen to the ortho-para mixture even at room temperatures. Still less, therefore, might one expect it to function as an agent for the production of para-hydrogen in the lower temperature range. In order, however, to test this further, since the data of Benton and White (71) showed activated adsorption of hydrogen at temperatures as low as $-110°C.$, experiments have been carried out by Mr. A. Sherman and the writer (76) with a form of nickel (10 per cent nickel on kieselguhr) known to possess an extreme activity for hydrogenation reactions and also showing specific adsorptions for hydrogen of a much higher order of magnitude than the material employed by Benton and White. It was obvious from the data already presented for manganous oxide and manganous-chromium oxide that it might be necessary to go to such nickel preparations of high activity to secure a sufficiently rapid velocity of adsorption and desorption of hydrogen at the low temperatures required for para-hydrogen formation. Our experiments at the temperature of liquid air were completely successful and it was found

possible to produce the equilibrium concentration of 50 per cent para-hydrogen as well on such an active nickel surface as on charcoal. That the activity of the surface was the important factor was readily shown by other experiments with small fragments of nickel wire as the surface material. With such, at liquid air temperatures, there was no measurable para-hydrogen formation. Again, therefore, we conclude that, at surfaces of high activity for surface reactions, we have high velocity, or, alternatively, low activation energy of the activating adsorption.

It is thus apparent that the spin isomerization of hydrogen may be utilized as an index to the nature of the adsorption process occurring at various surfaces at different temperatures. One example must suffice to establish the concordance thus anticipated between the data derived from velocity of adsorption and the isomerization process. It is evident from our data on oxide surfaces that the activating adsorption occurs in a higher temperature range than on the metals which show hydrogenation activity. This is true also of spin isomerization on a zinc oxide of high hydrogenation activity at higher temperatures, prepared by controlled ignition of zinc oxalate. With such zinc oxide, we obtain no para-hydrogen formation at liquid air temperatures although there is undoubtedly marked adsorption of hydrogen, presumably in the molecular unactivated form. At higher temperatures, from 0°C. upwards, the efficiency of the surfaces in the isomerization process can be tested by observing the extent of reconversion of a 50 per cent ortho-para mixture prepared over charcoal at liquid air to the normal 3:1 ortho-para mixture. With the same sample of zinc oxide, it has been found that the reconversion is barely perceptible after 15 minutes contact at 20°C., is definitely measurable after the same time at 50°C., is marked at 80°C., and is 80 per cent complete with the same time of contact at the temperature of boiling water. The measured velocity of reconversion increases exponentially with temperature. It may be that this represents actually the variation of the velocity of desorption of activated hydrogen from the zinc oxide surface. The velocity of desorption is slower than the velocity of adsorption since the former is proportional to $e^{-(E+Q)/RT}$

when the latter is a function of $e^{-E/RT}$, E being the activation energy of adsorption and Q the heat of adsorption. With zinc-chromium oxide, an even more active surface than zinc oxide, there is no para-hydrogen formation at liquid air temperatures even with 14 hours of contact time, but the reconversion of para- to the ortho-para-mixture is complete after 15 minutes contact at room temperature. This utilization of the para-hydrogen conversion for the purpose of determining the activity of hydrogenating surfaces is an interesting example of the applicability of
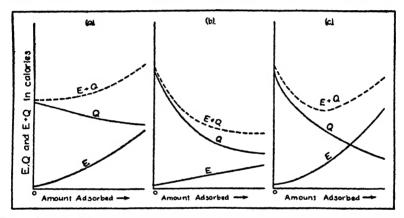
Fig. 1. Diagrammatic Representation of Activation Energy of Adsorption (E), Heats of Adsorption (Q), and Activation Energy of Desorption ($E + Q$), on Heterogeneous Surface

knowledge gained in abstract theoretical science to the practical problems of applied science. It is one more excellent illustration of the interdependence of theory and practice.

Activation energy of desorption

It is of interest to consider briefly the desorption of a molecular species which is adsorbed with energy of activation on a heterogeneous surface. On a homogeneous area, if the activation energy is E and the heat of adsorption is Q, the rate of desorption is proportional to $e^{-(E+Q)/RT}$. On a heterogeneous surface we have seen that, on the most active areas, the activation energy is least and that it increases steadily with increase of surface

covered. On the contrary, data on the heats of adsorption of gases on heterogeneous surfaces show high initial heats and steadily diminishing values with increased area of surface covered. Diagrammatically, the E and Q values vary as shown in figure 1. The algebraic sum of these magnitudes gives the activation energy of the desorption process for different portions of the surface area. These are indicated by the dotted lines in the diagram. It is apparent that, depending on the variations of E and Q relative to one another, various forms of the $E + Q$ curve may be obtained. The three indicated show (a) an $E + Q$ curve increasing with area covered, (b) one which decreases continuously, and (c) one showing a minimum. It is apparent that, from a surface corresponding to type (a), the gas would be desorbed first from the areas most active in adsorption. From a surface of type (b) the gas last adsorbed would be the first to be desorbed. In type (c), desorption would occur first from the areas of medium adsorption activity. There are at present no experimental data for E and Q for an actual surface so that nothing is known concerning the particular areas from which desorption most readily occurs. It is evident, however, that such areas would be the important areas in those surface-reactions where the velocity of desorption was the controlling rate of the reaction process.

Specific surface action

Consideration will show that the possibility of adsorption at rates governed by a definite activation energy must be of fundamental importance in those cases of specific surface action which have been so puzzling a feature of the general subject of reactions at surfaces. It is well known that, by a suitable choice of surface, a given reactant may be made to undergo one or another of several alternative modes of reaction, in some cases even at comparable temperatures. There has been much discussion of the possible factors which might determine the direction of reaction along a particular path. Hitherto, however, the possibility that the rate of activating adsorption or desorption might determine the path followed has not been considered.

We can conveniently illustrate the possibilities in this respect by reference to the alternative modes of decomposition of primary alcohols at various surfaces. It is well known that, at many metal surfaces and at the oxide surfaces already discussed for their hydrogenating-dehydrogenating activity, such alcohols decompose to form aldehydes and hydrogen. At the surfaces of other oxides already discussed in a preceding section, notably alumina, silica, thoria and the blue oxide of tungsten, the decomposition of the alcohols yields, practically exclusively, the corresponding olefin and water. The direction of the change which one and the same molecule undergoes is determined in major part by the chemical nature of the reaction surface. It has already been pointed out in an earlier section that there exists a remarkable parallelism between the capacity of surfaces to induce dehydrogenation or dehydration and the efficiency of such surfaces in promoting the recombination either of hydrogen atoms or of hydrogen atoms with hydroxyl radicals. This discovery by Taylor and Lavin (36) at once focusses attention on the final steps in the dehydrogenation and dehydration of alcohols which must consist in the recombination of the respective atoms or radicals at the surface and the evaporation of hydrogen of water. These final stages are the initial stages in the activating adsorption of hydrogen or water if the activation involved in such adsorption consists in a dissociation of the molecule. On this view, activity in dehydrogenation at a given temperature should be associable with an activated adsorption of hydrogen in a somewhat lower temperature range since, in general, desorption will occur more slowly than the adsorption and hence, for a given velocity, require a higher temperature. We have seen in the preceding pages that such hydrogen activation occurs on metals and certain oxides at temperatures which would permit the dehydrogenation reactions in question to occur fairly readily at temperatures of 200°C. and upwards. With surfaces such as alumina, active almost exclusively as dehydration agents at temperatures around 200°C., it is apparent that, from the point of view here developed, there should be no activating adsorption of hydrogen gas.

Experiment has confirmed the correctness of this conclusion and has led to an extension of the temperature range over which these processes of adsorption of hydrogen with activation energy have been measured. Some recent experiments of the writer (77) have shown that an active dehydration catalyst, composed of alumina, prepared by precipitation of the hydroxide by ammonia from aluminium nitrate and ignition at 400°C., shows no measurable adsorption of hydrogen below 400°C. At 440°C. a slow adsorption of hydrogen was recorded and the velocity of adsorption was measured. The velocity of adsorption was considerably increased by raising the temperature to 525°C. From the velocity data, an activation energy of 28,000 calories was deduced. The adsorption was a reversible process, the hydrogen being evolved and removable by evacuation at a higher temperature. No water was formed by reduction of the oxide. It is, therefore, obvious from such experiments that dehydrogenation does not occur on alumina surfaces at temperatures of about 200°C., which are normal for such decomposition processes because of the inability of such surfaces at such temperatures to effect the activation of hydrogen or the reverse recombination of hydrogen atoms. It is apparent also that water vapor must undergo an activating adsorption at temperatures in the neighborhood of those at which dehydration occurs. A test of this point on the lines so fruitful in the case of hydrogen would form a useful starting point for a generalization of the subject of adsorptions with activation energies. There seems to be every reason for concluding that water vapor may be adsorbed on alumina in two different forms, at low temperatures as unactivated molecules, and at the higher temperatures in that activated form in which it is effective in promoting hydration processes and from which it changes to a normal water molecule by desorption in dehydration processes. In extension of this thesis it may also be pointed out that Alyea (40) has shown a similar increase in the adsorption of hydrogen by powdered Pyrex glass from immeasurably small values in the low temperature range to values as high as 30 cc. in the temperature range of 480° to 520°C. The adsorption was reversible and showed the now familiar increase of velocity of

adsorption with temperature. As Alyea has elsewhere shown, this adsorption of hydrogen, setting in at elevated temperatures, is an important factor in the problem of the stationary and explosive reactions of hydrogen and oxygen in glass vessels, initiated, as shown by Alyea and Haber, at the glass surfaces. In this case, also, the temperatures at which the hydrogen is activated are much superior to those at which dehydration occurs.

What has been remarked here concerning the alternative processes of dehydrogenation and dehydration of alcohols may be adapted to other types of reaction and may serve as a useful method of approach to the whole field of specific surface activity. Thus, the nature of the association between the hydrogen halides and various halide surfaces is doubtless fundamental to the problem of addition of hydrogen halides to organic unsaturated compounds. There is evidence also in the recent work of Elgin (78) on the hydrogenation of different types of organic sulfur compounds that the most resistant sulfur compounds such as thiophene are activated only on the most active portions of the surface.

TEMPERATURE AND THE VELOCITY OF SURFACE REACTIONS

The velocity of surface reactions increases with temperature in the same manner as the velocity of homogeneous reactions, the variation with temperature being expressible by the well-known Arrhenius equation

$$d \ln k/dT = E_o/RT^2$$

The quantity E_o, which is now identified as the observed activation energy for the process in question, is, however, not always a simple magnitude but is normally composite of several energy quantities. This arises from the nature of the surface process. The effect of temperature extends not only to the actual interaction of the reaction species, but also to the variation of the concentrations of these species in the surface area which is the reaction volume of the process. Since these concentrations in the surface area are determined by adsorption, the influence of tem-

perature on the adsorbability of both reactants and products may become an important fraction of the total effect of temperature on the reaction. In one important class of surface reactions the observed activation energy, E_o, is actually identical with the true activation energy, E_t, of the surface process. In reactions which conform to the criteria of zero order reactions already discussed, the observed activation energy is equal to the true. This is so because of the nature of reactions of zero order. These occur only when the extent of the reaction surface covered with reactants does not sensibly change with external concentration, when the surface is saturated. If, in a given temperature interval, this condition also holds, there is then no variation in adsorption with temperature and hence no effect of adsorption on the observed temperature coefficient or activation energy. It is interesting to record in two of such zero order cases studied—hydrogen iodide decomposition on gold, $E_o = 25,000$ calories (16) and ammonia decomposition on tungsten, $E_o = 39,000$ calories (78a)—that the observed and therefore the true activation energies of the surface processes are materially less than the possible activation energies of any process of homogeneous decomposition. The surface process therefore involves a smaller increment of energy of the reactant molecules over the average energy of the system required for reaction to occur than would be required in the homogeneous process. The surface process can, therefore, be achieved at a lower temperature. Only in such cases is a surface reaction readily noticeable. When the reverse is true, the reaction occurring on the surface with the higher necessary increment of energy is lost in comparison with the homogeneous reaction. Examples of this kind are not experimentally available. We can, however, state the existence of one such. The homogeneous reaction of hydrogen and oxygen will overwhelm the surface reaction at oxidized tungsten surfaces for reasons already dealt with in preceding sections.

When adsorption of reactants and products at the surface varies with temperature a wide variety of relations between the observed and true energies of activation are possible, too many to reproduce here. It has been shown, however, by Polanyi

(79), Hinshelwood (19) and others that certain simple relations connect the two activation energies and the heats of adsorption of the several molecular species. Thus, for a single reactant, slightly adsorbed ($\sigma \propto p$), the observed and true activation energies are related by the expression,

$$E_o = E_t - \lambda_\mathrm{A}$$

where λ_A is the heat of adsorption of the reactant. If, in such a process, a product of the reaction is strongly adsorbed the relation becomes more complicated,

$$E_o = E_t - \lambda_\mathrm{A} + \lambda_\mathrm{B}$$

where λ_B is now the heat of adsorption of the retarding product. This latter equation is also applicable to such a case as a bimolecular reaction in which one of the reactants, B, is so strongly adsorbed that it leads to the kinetic equation

$$- dp/dt = k\,\frac{p_\mathrm{A}}{p_\mathrm{B}}$$

This is true in the case of the combination of hydrogen (A) and ethylene (B) on copper, studied by Pease (5), and for which an activation energy at ordinary temperatures of 10 kg-cal. was found. Since actual experimental data indicate that λ_A is about 10 kg-cal. and λ_B is about 16 kg-cal., it is evident that

$$E_t = 10 + 10 - 16$$

$$= 4 \text{ kg-cal.}$$

or less than half the observed activation energy. This activation energy is also very materially less than the energy necessary for the homogeneous process, which cannot be less than 30 kg-cal. and begins to occur only around 400°C. This example must also suffice to indicate that there is no essential correlation between the observed energy of activation and observed heats of adsorption, although this has been very recently considered by Maxted (80) as a possible method of approach to the problem of specific surface action.

One aspect of this relation between true and observed activation needs emphasis. Equations of the types just discussed are applicable only when the adsorption processes involved are rapid as compared with the actual reaction process proper. They cannot hold rigorously when the adsorption processes are relatively slow. It is evident from the discussion of the preceding section dealing with the velocity of adsorptions accompanied by an activation, in which it has been shown that such adsorptions may, indeed, be very slow, that particular attention must in future be paid to this condition attaching to any relation between true and observed activation energies on the one hand and heats of adsorption on the other. This factor may account for a discrepancy existing between two measurements of the activation energy of decomposition of isopropyl alcohol on bauxite studied by Dohse and Kälberer (8). By one experimental method, these authors studied the process occurring as a zero order reaction and obtained, therefore, a true activation energy of 26,000 calories. When studied as a unimolecular decomposition inhibited by the product, water, they observed an activation energy of 39,000 calories. To derive a true activation energy from this, use must be made of the equation previously developed which, when applied to this special case, has the form

$$E_t = E_o + \lambda_{C_3H_7OH} - \lambda_{H_2O}$$

Kälberer and Dohse measured the heats of adsorption and found $\lambda_{C_3H_7OH} = 21,000$ calories and $\lambda_{H_2O} = 13,000$ calories. Hence, from the unimolecular inhibited reaction one derives a value, $E_t = 39,000 + 21,000 - 13,000 = 47,000$ calories in sharp disagreement with the result, 26,000 calories, from the zero order reaction. The discrepancy is even more conspicuous when we note that water, with $\lambda = 13,000$ calories, is inhibiting the access of isopropyl alcohol, with $\lambda = 21,000$ calories, to the surface. It is evident that, to obtain agreement between the data for true activation energy in the zero order and unimolecular reactions, it would be necessary to employ a value of $\lambda_{H_2O} = 34,000$ calories in the equation

$$E_t = E_o + \lambda_{C_3H_7OH} - \lambda_{H_2O}$$

Now such a value is reasonable if, instead of the measured heat of adsorption of water (λ_{H_2O} = 13,000), we substitute an assumed heat of desorption of water, λ_{H_2O} = 34,000 calories. Granting that the measured heat of adsorption is correct, this would involve an energy of activation of water at a bauxite surface equal to 34,000 − 13,000 = 21,000 calories. While this appears to be somewhat high, it is of the right order of magnitude. There is the distinct possibility, also, that the heat of adsorption of water actually measured by Dohse and Kälberer is too low, involving a measurement of a heat effect of adsorption only part of which was an activated adsorption. We have already seen in the case of hydrogen on manganous-chromium oxide catalyst that the data of Williamson and the writer for unactivated and activated adsorption are respectively about 2000 calories and >19,000 calories. The presumption is, therefore, that the measured value for λ_{H_2O} = 13,000 calories is in reality too small and that an energy of desorption λ = 34,000 calories would be a closer approximation to the correct value to be employed.

What has been stated in particular for this reaction can be extended quite generally to reactions in which one of the reactants behaves as a retardant. This suggests that a degree of special importance would attach to studies of kinetics and of adsorption data for dehydrogenation reactions at oxide surfaces for which the data already presented here indicate activated adsorptions and to readily available methods of separating such activated adsorptions from the molecular adsorptions of hydrogen occurring in another temperature range. The existence, side by side, of activated and unactivated adsorption at a variety of surfaces causes a degree of doubtfulness to attach to all the measurements of heats of adsorption hitherto made in this field (8, 64, 65, 68, 69, 81). The data for hydrogen on the metals are least suspect since, apparently, the activated adsorption occurs even at quite low temperatures. The recognition of the two possible types of adsorption must, however, lead to a greater discrimination in the future in the choice of experimental procedures.

For unretarded reactions, another factor of importance in connection with the observed activation energy is the hetero-

geneity of the surface.   Normally, with a heterogeneous surface, the heat of adsorption diminishes continually with increase of surface covered.   In a reaction in which $E_o = E_t - \lambda$, we may write the velocity of reaction

$$- dp/dt = z \cdot e^{-(E_t - \lambda)/RT}$$

where z is a collision factor between the reactant and surface. On two equal surface areas on which the z factor is constant but on which the heats of adsorption are respectively λ_1 and λ_2, the rates on the two areas will be, if E_t be assumed constant,

(1) $z\, e^{-(E - \lambda_1)/RT}$

and

(2) $z\, e^{-(E - \lambda_2)/RT}$

The ratio of these two rates is obviously $e^{-(\lambda_2 - \lambda_1)/RT}$.   For two areas on which $\lambda_1 - \lambda_2 = 5000$ calories this corresponds to a ratio of rates equal to $e^{5000/RT}$ or, at $T = 500°K.$, e^5 or 158.4. This means that the reaction on area 2 would be quite negligible as compared with that on area 1.   If E_t varies from area to area the ratio of the reaction rates would be

$$r_1/r_2 = e^{-[(E_{t_1} - E_{t_2}) - (\lambda_1 - \lambda_2)]/RT}$$

Since there is evidence in available experimental material that the true activation energy increases with decreased activity of this surface, it follows that this ratio is even greater than that obtaining when E_t is constant.   These ratios indicate very definitely the possibility of areas of the surface to which reaction is very largely confined and other areas on which only a negligible amount of reaction occurs.   It is this effect of heterogeneity in surfaces which makes pronounced the effect of minimal amounts of poisons on surfaces.   It is such heterogeneity which the use of promoters emphasizes.   Conversely, it may be stated that the existence of pronounced poisoning by minimal amounts of materials or marked promotion of surface activity by small amounts of added agents is a direct index of the existence of a heteroge-

neity of surface on the most active areas of which alone is the reactions in question markedly achieved.

Such then is one record of progress in the study of reactions at surfaces. To the writer it appears as one in which there has been a steady development away from empiricism and towards a scientific analysis of phenomena which, for too long a period of time, lay hidden under the cloak of a nomenclature which served also to conceal ignorance and arrest progress. The record is not one of finality—indeed, it can be but the prelude to a more illuminating sequel in which those who read may be inspired to share, for "what I now chance to approve, may be or become to others strange and unpalatable" since "this picklock Reason is still a-fumbling at the wards."

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THE NATURE OF THE ADSORBED PHASE

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Surface phenomena are so complex that it is almost imperative to group them for discussion into sections. It has been found convenient to deal first with the kinetics of adsorption, then with the equation of state of a film, and finally with electrical properties of the adsorbed phase. The discussion will mainly be limited to the adsorption of a gas on a solid.

I. KINETICS

The condensation of molecular streams

The conditions controlling the condensations of molecular streams were first studied by Wood and Knudsen (1). They found that there was a critical surface temperature above which an atomic beam (composed for example of cadmium atoms) would not condense on glass, but would be reflected in a diffuse manner. Langmuir (2) pointed out that another interpretation could be given to these results by supposing that condensation occurred at all temperatures, followed by evaporation after a mean time which was a function of the temperature. This would account for the diffuse reflection at the higher temperatures. But later work has shown that the interaction between atomic beams and the surface is more subtle than was suspected. In some cases the beam acts as would a train of de Broglie waves of wave length $\frac{h}{mv}$ (h is Planck's constant, m the mass of the atom, v its velocity).

Thus, Ellett, Olson and Zahl (3) showed that cadmium is reflected specularly from a sodium chloride crystal, with a velocity selection in the reflected beam. Here the reflection was that for a

space grating. Knauer and Stern (4) found that helium and hydrogen beams are diffracted by sodium chloride and potassium chloride as by a surface grating; Esterman and Stern (5) found a similar result for the reflection of the same gases from lithium fluoride; and Johnson (6) found that atomic hydrogen produces a reflection pattern from lithium fluoride satisfying cross-grating formulas. On the other hand, no specular reflection was found for sodium impinging on sodium chloride (7), or for lithium, potassium, and cesium on sodium chloride (8) and lithium fluoride. Similarly cadmium and arsenic are not reflected specularly from orthoclase or fluorite. It seems strange also that heavy atoms such as cadmium should penetrate through several layers, while lighter atoms are reflected by a surface grating. There is thus no general rule at present governing the reflection of atoms.

However, Langmuir's contention, requiring as it does the dependence of critical temperature on stream density, has been supported by the experiments of Chariton and Semenow (9), Esterman (10), and Cockroft (11), and forms the basis for the more elaborate analysis of Frenkel (12). Before passing on to the latter it will be well to discuss the adsorption time or mean life of a molecule on a surface.

The mean life of an adsorbed molecule

Suppose that νdt molecules fall on a surface S in time dt and that a fraction ρ is reflected. Then $(1 - \rho)\nu dt$ molecules are adsorbed in time dt: call this number b_0. We wish to know the number b of these b_0 molecules which are left on the surface after time t.

If $w\, dt$ is the probability that a molecule leaves the wall in time dt, then

$$- db = b\, w \cdot dt \quad \text{and} \quad b = b_0\, e^{-wt}$$

The mean life τ of the molecules which are adsorbed is therefore

$$\frac{1}{b_0} \int_{b_0}^{0} - db \cdot t = \frac{1}{w}$$

The mean adsorption time of the molecules falling on the surface is

$$\tau_1 = (1 + \rho)\,\tau$$

On this basis, Clausing (13) derives Langmuir's familiar adsorption isotherm. Let us suppose that in the stationary state there are n molecules per area S: then $n = \nu\tau_1$. Let there be N' adsorption points on the surface, and let us suppose that a molecule hitting an adsorbed molecule is reflected, whereas one hitting a free spot is adsorbed with probability β. Then in the stationary state

$$\rho = \frac{n}{N'} + (1 - \beta)\left(1 - \frac{n}{N'}\right)$$

and hence,

$$\tau_1 = \left(1 - \frac{n}{N'}\right)\beta\,\tau$$

Since $n = \nu\,\tau_1$, this gives

$$n = \nu\left(1 - \frac{n}{N'}\right)\beta\,\tau, \qquad n = \frac{\nu\,\beta\,\tau\,N'}{\nu\,\beta\,\tau + N'}$$

which is Langmuir's isotherm. Here $\beta\tau$ is the mean life of a molecule falling on the free surface. Langmuir took β to be unity.

Clausing calculates from Langmuir's data for the adsorption of gases on glass the following values for $\beta\tau$ at $90°$K.: argon, 1.9×10^{-5}; nitrogen, 2.27×10^{-5} and 5×10^{-5}; oxygen, 1.4×10^{-5}; carbon monoxide, 30.8×10^{-5}. Wertensein (14) found for mercury on glass at $21°$C. that $\tau_1 = 1.08 \times 10^{-5}$.

Clausing has also measured the mean life of an adsorbed molecule directly. A vertical molecular stream was directed against a rotating horizontal plate. The molecules adsorbed on the plate were carried along by it, given off, and condensed on a cooled surface. The distance of the latter deposit from the point of

impact allowed the adsorption time to be measured. At 200°K.
an upper limit of 10^{-6} was obtained for cadmium on various
surfaces. Later, Clausing made more precise measurements by
allowing a molecular stream at low pressure to pass through a
glass capillary into an evacuated space. He thus found the
time for a molecule to pass through the capillary. For argon
on glass between 78° and 90°K. he found that τ_1 was given by
$1.7 \times 10^{-4} e^{\frac{3800}{RT}}$ (at 78°K., $\tau_1 = 75 \times 10^{-5}$; at 90°K., $\tau_1 = 3.1 \times 10^{-5}$). These figures are sufficiently close to Langmuir's.
Neon gave between 78° and 90°K. a value smaller than 2×10^{-7}.

The analysis of Frenkel

A variation with temperature of the adsorption time such as
was found by Clausing is readily intelligible in terms of Fren-
kel's theory (12). As before, let us suppose that we have n
atoms on an area S and that $n = \nu \tau_1$. If an adsorbed molecule
executes an S.H.M. of period τ_0 perpendicular to the surface,
the potential energy at a displacement z is given by

$$\Delta u = \frac{2 \pi^2}{\tau_0^2} \cdot m z^2$$

where m is the mass of the molecule, giving the total energy of a
displaced molecule the value $- u_0 + \Delta u$, where u_0 is the energy
in the equilibrium position. The thickness of the adsorption
region, δ, may thus be defined by

$$\int_{-}^{\infty} e^{-\frac{\Delta u}{kT}} dz \qquad \tau_0 \sqrt{\frac{kT}{2 \pi m}}$$

Since

$$Sc \sqrt{\frac{kT}{2 \pi m}}$$

where c is the bulk concentration, and

$$\frac{n}{S} = c \cdot \delta \cdot e^{\frac{u_0}{kT}}$$

for small surface concentrations, i.e., neglecting the area occupied by the electron atmospheres of the molecules, we obtain

$$\tau_1 = \tau_0 \, e^{\frac{u_0}{kT}}$$

Frenkel then proceeds to consider the formation of agglomerates. Langmuir (15) demonstrated the presence of these by allowing a stream of cadmium atoms to impinge on a cooled glass surface for so short a time that no visible deposit was formed. On warming to room temperatures the agglomerates had not evaporated, as single atoms would have done, for they could act as condensation nuclei for cadmium vapor.

Only paired atoms are considered. Let there be n_1 atoms singly, and n_2 atoms doubly linked on a surface S. Frenkel writes the probability that the distance of one molecule from another should not be greater than

$$\pi \, d_0^2 = \sigma_0,$$

where d_0 is the molecular diameter, as

$$(n - 1) \frac{\sigma_1}{S}$$

Hence

$$n_1 = n \left[1 - (n - 1) \frac{\sigma_0}{S} \right]$$

and

$$n_2 = n \, (n - 1) \frac{\sigma_0}{S} = \frac{n^2 \, \sigma_0}{S}$$

Owing to the attractive forces between atoms this number n_2 must be multiplied by $e^{\frac{\Delta u}{kT}}$, where Δu is the dissociation energy of a doublet: we may write

$$\sigma = \sigma_0 \, e^{\frac{\Delta u}{kT}}$$

and

$$u_1 = u_0 + \Delta u$$

We may note that the law of mass action applied to the surface gives a similar expression. The surface concentrations are $\dfrac{n_1}{S}$ and $\dfrac{n_2}{S}$. Hence

$$n_2 = \frac{n_1^2}{S} \cdot K = \frac{n_1^2}{S} \cdot A \, e^{\frac{\Delta u}{kT}} = \frac{n^2}{S} \cdot A \, e^{\frac{\Delta u}{kT}}$$

for small n. A more accurate statistical mechanical treatment may also be applied. The free energy f_1 of a two-dimensional gas may be written

$$e^{\frac{-f_1}{kT}} = \frac{e^{\frac{-E}{kT}}}{n_1 \, h^2} \cdot 2 \, \pi \, m \, k \, T \cdot S$$

where m is the mass of a molecule. Allowing for rotation and interatomic vibration of the double molecule we can calculate the equilibrium constant as for a three-dimensional dissociation (16). This gives

$$n_2 = \frac{n_1^2}{S} \cdot \left(\frac{\pi \, k \, T}{m} \right)^{\frac{1}{2}} \cdot \frac{d_0}{\nu_0} \cdot e^{\frac{\Delta u}{kT}}$$

where ν_0 is the interatomic vibration frequency. In practice the inclusion of the power $T^{\frac{1}{2}}$ would have practically no effect. Hence the equilibrium equation becomes

$$\frac{dn}{dt} = \nu - w_1 n - w_2 n = 0$$

where the w's are as above, giving

$$- n = \frac{(w_1^2 - 4 \, \beta' \, \nu)^{\frac{1}{2}} + w_1}{2 \, \beta'}$$

where

$$\beta' = (w_1 - w_2) \frac{\sigma}{S}$$

Since the discriminant must be positive

$$\nu > \frac{w_1^2}{4\,\beta'}$$

The critical stream density is therefore

$$\nu = \frac{w_1^2}{4\,(w_1 - w_2)} \cdot \frac{S}{\sigma}$$

Hence

$$e^{\frac{u_1}{kT}}\left(1 - \frac{\tau_0}{\tau_s}\,e^{\frac{-\Delta u}{kT}}\right) = \frac{}{4}\,\sigma_0\,\nu\,\tau_0$$

where τ_s is the period of the oscillation perpendicular to the surface of an atom bound in a pair. Hence (approximately)

$$\frac{e^{\frac{u_1}{kT}}}{4\,\sigma_0\,\tau_0\,\nu}$$

It is convenient to regard the agglomerate as a pair. This is in accordance with the low values found for u_1 as compared with the latent heat of evaporation of a cadmium crystal.

Frenkel's relation for the critical stream density was experimentally verified by Cockroft, for the condensation of cadmium on copper. Over the range $-150°C.$ to $-90°C.$, the critical density varied with temperature according to

$$\nu = 4.7 \times 10^{12}\,e^{\frac{-2840}{T}}$$

Hence $u_1 = 5680$ calories per mole, whereas the latent heat of evaporation of cadmium crystal is 32,000 calories per mole. Cockroft found that the value of ν was the same for surfaces of copper, silver and glass, but that if a fresh silver surface were deposited immediately before condensation of the cadmium stream the density required to form a deposit decreased by a factor of ten. This shows that the specific surface forces do not normally come

into play, as the surface, without special precaution, is coated with a layer of adsorbed gas. In the same way the thermal accommodation coefficients $\left(\dfrac{T_1 - T_2}{T_1 - T_2'}\right.$, where T_1 is the temperature corresponding to the energy of a gas molecule approaching the surface, T_2 the temperature of one leaving the surface, and T_2' the surface temperature) are roughly independent of the nature of the surface.

The mobility of atoms in the surface phase

There is a good deal of experimental evidence to support the implication of Frenkel's theory that molecules can move about on the surface. Such a motion doubtless occurs in many cases on a cushion of adsorbed gas molecules.

Esterman (10) found that when V-shaped diaphragms were inserted in the path of the molecular stream no deposit occurred at the narrow part of the trace, when the surface temperature was just below the critical value for the stream density used. The critical density is therefore greater at the edge of a deposit than at a point inside, pointing to a lateral creep away from the edge.

Volmer and Adhirkari (17) were able to detect the motion of benzophenone over a glass surface. The benzophenone passed from a solid crystal over a strip of glass .01 cm. wide and was drawn off by mercury. The value 1.5×10^{13} dynes was calculated for the surface viscosity constant. Similarly, Volmer and Esterman, from the growth of mercury crystals in vapor at low pressure, came to the conclusion that free motion of adsorbed mercury molecules could occur over the surface.

Cockroft (11) placed a wire across his copper condensation surface at a very short distance from the surface, and allowed the beam from his oven to form the usual circular deposit. When the circle had reached a certain size the wire was moved to another position by an electromagnet. In this second position the region shadowed by the wire had been sensitized outside the circular deposit, and any molecules creeping in would be caught and held. It was found that when deposition was continued the shadow region outside the first circular deposit showed crystals of cadmium when examined under the microscope. These crystals had crept in from the sides.

The effect of a surface film of gas atoms on the reflection of electrons from a nickel surface was investigated by Germer (18). At room temperatures the results could be interpreted in terms of a regular two-dimensional lattice of gas atoms. On heating to 150°C. this lattice had melted to give an irregular arrangement.

There is no doubt that in such reactions as the reduction of cupric oxide by hydrogen (19), where the reaction region is the boundary between copper and cupric oxide, the rapidity of the reaction is due to the motion of adsorbed hydrogen over the surface to the reaction zone.

II. THE EQUATION OF STATE OF THE ADSORBED PHASE

The equation of state

Various lines of evidence justify the treatment of a surface film as a separate phase, with properties analogous to those of the bulk material. In particular, the lateral mobility of adsorbed molecules, the ease with which such a concept allows the relations with the bulk gas to be derived, and the direct observation of films on liquids, support the view that a surface film exerts a surface pressure which is related to the area of the film and the temperature by an equation of state. ·

The equation of state of a dilute gaseous film may readily be derived in an approximate form.   Consider a molecule of mass m and surface coördinates x, y, acted on by forces whose components are X, Y and by frictional resistances of the form $- k_1 (\dot{x} \, \dot{y})$. Then

$$m \, \ddot{x} = X - k_1 \, \dot{x}$$

Multiplying this by $\tfrac{1}{2}x$ this equation is the same as

$$\tfrac{1}{4} \cdot \frac{d^2}{dt^2} (mx^2) + \tfrac{1}{4} \cdot \frac{d}{dt} (k_1 x^2) - \tfrac{1}{2} mx^2 = \tfrac{1}{2} Xx$$

On adding this to the similar equation in y, and putting

$$x^2 + y^2 = r^2$$

we obtain

$$\tfrac{1}{4} \cdot \frac{d^2}{dt^2} (mr^2) + \tfrac{1}{4} \cdot \frac{d}{dt} (k_1 r^2) = \tfrac{1}{2} mv^2 + \tfrac{1}{2} (Xx + Yy)$$

This is now summed over all the molecules on the surface S and integrated over a long time t giving

$$\frac{1}{t} \cdot \left[\tfrac{1}{4} \cdot \frac{d}{dt} \cdot \Sigma \ (mr^2) + \tfrac{1}{4} \Sigma \ (k_1 r^2) \right]_0^t = \tfrac{1}{2} \ \overline{\Sigma mv^2} + \tfrac{1}{2} \ \overline{\Sigma Xx + Yy}$$

where $\overline{\Sigma mv^2}$ and $\overline{\Sigma Xx + Yy}$ are the average values over the time t. In the steady state the values of the expression in brackets are the same at times 0 and t. Hence we have the virial equation of Clausius

$$\tfrac{1}{2} \Sigma mv^2 = - \tfrac{1}{2} \Sigma Xx + Yy$$

where $-\tfrac{1}{2}\Sigma Xx + Yy$ is the virial.

Suppose that n molecules are enclosed in a surface S and exert a surface pressure F. The virial is made up of the intermolecular forces and the stresses across the boundary. If ds is an element of the latter, and l and m are the direction cosines of the outward normal to the boundary, the stress components are $-l \cdot F \cdot ds$, $-m \ F \cdot ds$, contributing to the virial

$$- \tfrac{1}{2} F \int (lx + my) \ ds = \tfrac{1}{2} F \cdot \int \int \left(\frac{\partial x}{\partial x} + \frac{\partial y}{\partial y} \right) \cdot dS$$

$$= FS$$

In order to obtain the contribution of the intermolecular forces to the virial, take two molecules whose centers are at x, y and x', y'. Let the force components be X, Y and X', Y', and the intermolecular force $-\dfrac{\partial E}{\partial r}$. Then

$$X = - \frac{\partial E}{\partial r} \cdot \frac{(x - x')}{r}$$

$$X' = - \frac{\partial E}{\partial r} \cdot \frac{(x' - x)}{r}$$

and hence

$$xX + x'X' = - \frac{\partial E}{\partial r} \cdot \frac{(x - x')^2}{r}$$

The contribution of the intermolecular force to the virial is therefore $\frac{1}{2} r \cdot \frac{\partial E}{\partial r}$, and hence

$$\frac{1}{2} \overline{\Sigma m v^2} = F \cdot S \cdot + \frac{1}{2} \Sigma r \frac{\partial E}{\partial r}$$

The average number of pairs of two molecules whose centers are at a distance between r and $r + dr$ of one another is

$$\frac{1}{2} n^2 e^{-E/kT} \cdot \frac{2 \pi}{S} \cdot r \cdot dr$$

for the number of pairs is $\frac{1}{2} n(n - 1)$ and the factor $e^{-E/kT}$ allows for the intermolecular force. Hence, putting

$$\frac{1}{2} \Sigma m v^2 = nkt$$

$$FS = nkT - \frac{n^2 \pi}{2 S} \cdot \int_0^\infty r^2 \cdot \frac{\partial E}{\partial r} \cdot e^{-E/kT} \cdot dr$$

On integrating this by parts we obtain the alternative form

$$FS = nkT - \frac{n^2 \pi kT}{S} \cdot \int_0^\infty r \left(e^{-E/kT} - 1 \right) \cdot dr$$

provided that

$$Lt_{r \to \infty} r^2 \left(e^{-E/kT} - 1 \right) = 0$$

$$FS = nkT - \frac{nkTB}{S}$$

where B is the second virial coefficient. When S is replaced by its approximate equivalent $\frac{nkT}{F}$ in the right hand side of this equation, we get the inverted form

$$FS = nkT - BF$$

or

$$F(S - B) = nkT.$$

When E is known as a function of r, B may be formulated more explicitly.   Putting

$$\frac{\partial E}{\partial r} = \frac{\lambda_q}{r^q} - \frac{\lambda_m}{r^m}$$

we can find B as Lennard-Jones (20) does for a three-dimensional gas.   This gives

$$B = \frac{n\,\pi}{2\,kT}\left(\frac{\lambda_q}{\lambda_m}\cdot\frac{m-1}{q-1}\right)^{\frac{2}{q-m}}\cdot F(y)$$

where

$$= \left(\frac{q-1}{\lambda_q}\,kT\right)^{q-1}\cdot\frac{\lambda_m}{kT\,(m-1)}$$

$$F(y)\quad y^{\frac{2}{q-m}}\left[\Gamma\left(\frac{q-3}{q-1}\right) - \sum_{t=1}^{\infty} f(t)\,y^t\right]$$

and

$$f(t) = \frac{}{\lfloor t\,(q-1)}\;,\left[\frac{t\,(m-1)-2}{q-1}\right]$$

Unfortunately the temperature variation of the second virial coefficient is not sufficiently well known even for thin films on liquids to enable the force constants of molecules to be calculated on this basis.

A gas equation of the form deduced above, i.e.,

$$F(S - B) = nkT,$$

was postulated by Volmer (21).   It has the merit of giving a simple derivation of Langmuir's isotherm.   Adopting the treatment of the author (22), the free energy, f, per molecule is

$$\int \frac{S}{n}\cdot dF = kT\log\frac{nkT}{S-B} + \frac{BkT}{S-B} + \text{constant}$$

Since the corresponding value for the bulk gas is $kT \log p +$ constant, at equilibrium

$$kT \log \frac{nkT}{S - B} + \frac{B \, kT}{S - B} = kT \log p + K_1$$

where K_1 is a constant, approximately independent of temperature. Hence

$$\log \frac{p(S - B)}{n} = \frac{B}{S - B} + K_2$$

and if $\dfrac{B}{S - B}$ is small compared with K_2,

$$\frac{n}{S} = p \, (K_2 + B \, p/n)^{-1}$$

which is Langmuir's isotherm (B is proportional to n). We note that at saturation $\dfrac{1}{S} = \dfrac{1}{B}$ and that

$$K_2 = kT \, e^{\phi/kT}$$

where ϕ is a constant. From this point of view the isotherm is an expression of the distribution law, if the effective concentration is taken to be $\dfrac{n}{S - B}$ for the surface phase.

The gas equation of surface films on liquids may be directly studied in certain cases, and it is interesting to compare the results with theory. The surface concentration of a capillary active solute may be deduced by Gibb's equation, and a relation found between this and the surface pressure

$$F = \sigma_0' - \sigma'$$

where σ_0' and σ' are the surface tensions of the pure solvent and the solution. Traube (23), for example, found that for small values of F,

$$FS = nkT$$

and the analogy between this and the equation of a perfect gas led him to regard the lowering of the surface tension of the solvent as the effect of the two-dimensional pressure. However, for larger F's this approximation no longer holds. Clearly

$$\frac{F}{kT}\frac{S}{n} = \frac{F}{kT} \cdot \frac{d f_2}{d \sigma'} = \frac{d (\log a)}{d (\log F)}$$

where f_2 is the partial molar free energy, and a the activity, of the solute. Assuming that activity and concentration were equal Rideal and Schofield (24) calculated $\dfrac{FS}{nkT}$ for water solutions of the lower fatty acids from Szyszkowski's data, and found that

$$F(S - B) = x'nkT$$

which is similar to the equation of simple theory, except for the factor x'. This equation is in fact the analogue of Amagat's equation for a gas at high pressure. Jones and Bury (25) have pointed out that in these solutions concentration and activity diverge widely, and find that for high concentrations of solute the Gibb's surface excess remains a constant with increasing concentration, instead of decreasing, the result obtained when activity is replaced by concentration. This, however, scarcely affects the form of the gas equation deduced from measurements at low concentrations.

Similar results are obtained with insoluble films on water: Adam and Jessop (26) found that the equation of state of gaseous films was the analogue of Amagat's equation. It would be interesting in this connection to examine binary mixtures for departures from additivity. We have

$$\frac{F}{kT} = \frac{n_1 + n_2}{S} - \frac{1}{S^2} [\tfrac{1}{2} \rho_{11} n_1^2 + \rho_{12} n_1 n_2 + \tfrac{1}{2} \rho_{22} n_2^2]$$

where

$$\rho_{\alpha\beta} = 2 \pi \int_0^\infty (e^{-E_{\alpha\beta}/kT} - 1) \, r \cdot dr$$

Putting $n_1 = x\,n$, and $n_2 = (1 - x)n$, we get

$$\frac{F}{kT} = \frac{n}{S} - \tfrac{1}{2}\frac{n^2}{S^2}\left[\rho_{11}\,x^2 + 2\,\rho_{12}\,x(1 - x) + \rho_{22}\,(1 - x)^2\right]$$

so that a linear dependence on x, the mole fraction, would be exceptional (27).

The adsorption isotherm

We should expect that the actual equation of state of a surface layer would be the analogue of Onnes' equation for a bulk gas

$$pV = A + \frac{B}{V} + \frac{C}{V^2} + \frac{D}{V^4}\cdots\cdots$$

where the coefficients A, B, etc. vary with temperature. The equation of Beattie and Bridgman (28) has the merit of giving a virial development in terms of only five constants. The equation runs

$$P = \frac{RT}{V} + \frac{RT}{V^2}\left(B_0 - \frac{C}{T^3} - \frac{A_0}{RT}\right) + \frac{RT}{V^3}\left(\frac{c\,B_0}{T^3} - b\,B_0 + \frac{a\,A_0}{RT}\right) - B_0\,\frac{c\,b\,RT}{V^4\,T^3}$$

$$= \frac{RT}{V} + \frac{RT}{V^2}\,\beta + \frac{RT}{V^3}\,\gamma + \frac{RT}{V^4}\,\delta$$

where A, B, a, b, c are constants, V the gram-molecular volume, and β, γ, and δ vary with temperature as shown. The free energy takes the form

$$\int Vdp = RT\left[-\log V + \frac{2\,\beta}{V} + \frac{3\,\gamma}{2\,V^2} + \frac{4\,\delta}{3\,V^3}\right] + \text{constant},$$

and, analogously, for a surface phase

$$\int A\,dF = RT\left[-\log A + \frac{2\,\beta}{A} + \frac{3\,\gamma}{2\,A^2} + \frac{4\,\delta}{3\,A^3}\right] + F_0$$

where A is the area per gram-molecule.

Bradley (29) has used this equation to study Langmuir's isotherm. If z is the number of gram-molecules adsorbed per unit area, $z = \dfrac{1}{A}$,

$$\log p + \frac{F_0{}'}{RT} = \log z + 2\beta z + \tfrac{3}{2}\gamma z^2 + \tfrac{4}{3}\delta z^3 + \frac{F_0}{RT}$$

This is the adsorption isotherm, and it remains to link it with Langmuir's. The latter may be written

$$z = \frac{c_1 c_2\, p}{1 + c_2\, p} \quad \text{or} \quad p = \frac{z}{c_1 c_2 - c_2\, z}$$

Hence

$$\log p = \log z - \log (c_1 c_2 - c_2\, z)$$

$$= \log z - \log c_1 c_2 + \frac{z}{c_1} + \frac{z^2}{2\, c_1{}^2} +$$

since z is always less than c_1. This is indeed the theoretical form, provided

$$\frac{1}{c_1} = 2\beta = 2\left(B_0 - \frac{C}{T^3} - \frac{A_0}{RT}\right), \text{ and } \frac{\Delta F_0}{RT} = -\log c_1 c_2$$

These two equations are in fact approximately obeyed. The work of Zeise (30) has shown that the adsorption of gases on charcoal can be represented better in terms of Langmuir's theory than by means of Polanyi's adsorption potential. He finds that the parameters c_1 and c_2 vary with temperature according to

$$c_1 = a_1 - b_1\, T \qquad c_2 = \frac{10^{a_2}}{T^{b_2}}$$

The results deduced above are equivalent to these two equations, for, taking the case of carbon dioxide, for which there are most points, c_1 can be expressed by

$$\frac{1}{c_1} = -.509 + \frac{15.9}{T} + \frac{3 \times 10^5}{T^3}$$

while the plot of $\log c_1 - c_2$ against $\dfrac{1}{T}$ is a straight line except at low temperatures: ΔF_0 is regarded as approximately invariant with temperature. We may note that Zeise's equation for c_1 is theoretically inadequate in that it predicts a negative adsorption at high temperatures.

The scope of Langmuir's isotherm thus appears to be considerably wider than was supposed, and the temperature variation of the coefficients can be accounted for in general terms. More recently Langmuir's simple kinetic treatment has been replaced by statistical derivations. Frenkel introduced his result

$$\tau = \tau_0\, e^{\frac{u_0}{kT}}$$

into the isotherm in the form given in Section I, viz.,

$$n = \frac{\nu\,\beta\,\tau\,N'}{\nu\,\beta\,\tau + N'}$$

when $\beta = 1$. Writing

$$Sp$$

we get

$$\frac{N'}{n} = 1 + \frac{N'}{S}\,\frac{\sqrt{2\,\pi\,mkT}}{p\,\tau_0}\cdot e^{u_0/kT}$$

which is Frenkel's isotherm if $\dfrac{S}{N'}$ is replaced by σ_0. We note that $c_1 c_2$ is proportional to

$$\frac{\tau_0}{T^{\frac12}}\cdot e^{-u_0/kT}$$

The same result may be derived by writing the distribution law between the phases of three and two dimensions in the form

$$S - n\,\sigma_0 \qquad \frac{p\,\tau_0}{}\cdot e^{u_0/kT}$$

Kar (31) has deduced the equation

$$F(S - B) = nkT$$

statistically.   He finds that Langmuir's equation holds with

$$c_1 c_2 \quad \frac{kT}{\rho} \cdot \sqrt{2\pi mkT} \cdot e^{-u_0/kT}$$

where ρ is a multiple of Planck's constant h.   This result is equivalent to Frenkel's if the oscillation of the adsorbed molecule is quantized, for then (32)

$$\frac{m_1 h}{kT}$$

Hückel (33) writes

$$c_2 = v\, e^{u_0/kT}$$

where u_0 is the adsorption potential, and v the active volume of an adsorbed molecule.   Since v, according to Herzfeld (34), is equal to

$$\frac{1}{(2\pi mkT)^{\frac{3}{2}}} \left(\frac{kT}{\nu}\right)^3$$

we obtain the result

$$c_2 \propto \frac{1}{T^{\frac{3}{2}}} \cdot e^{u_0/kT}$$

if the oscillation is quantized.

Analogies between phases of two and three dimensions .

Reference has frequently been made in this article to the striking analogy between a surface phase and a bulk material.   Rideal and Lyons (35) have pointed out that for films on liquids the various types of film—solid condensed, liquid condensed, liquid expanded, and vaporous—are analogous to the bulk phases—

solid, liquid crystal, liquid, and vapor. The pressure-temperature curves delimit phase regions for films exactly as for the bulk phases, so that the films show a triple point. Various thermal constants may be calculated, such as the two-dimensional latent heat of evaporation. There is thus the possibility of the coexistence of two or more surface phases, separated by the boundary regions, in this case lines. There will be adsorption at these lines according to an equation analogous to that of Gibbs, and these lines may themselves be treated as separate regions, with an equation of state (Schwab and Pietsch (36)). We may refer to linear just as to surface energy, and indeed the former has been calculated for the edges of certain salts. These lines, which are regions of greater activity than the rest of the surface, are clearly of importance to heterogeneous catalysis, especially when it is remembered that actual crystals are composed of small units separated by cracks.

Many of these analogies have not been exploited. It would be interesting, for example, to study the homogenous reaction between two components of a surface phase.

III. ELECTRICAL PROPERTIES OF THE ADSORBED LAYER

General remarks on polarization

From the viewpoint of dielectric theory (37) molecules fall into two types. In the one the molecular polarization is a constant; in the other it splits up into two parts, one of which is a constant, while the other varies inversely as the absolute temperature. The second type is explained by assigning to the molecule a permanent electric doublet, μ. This is found by classical statistics to contribute $\dfrac{4\pi N\mu^2}{9kT}$ to the molecular polarization, which is therefore given by

$$\frac{\epsilon - 1}{\epsilon + 2} \cdot \frac{M}{d} = \frac{4}{3} \cdot N \left(\alpha + \frac{\mu^2}{3 \, kT} \right)$$

Here ϵ is the dielectric constant, M the molecular weight, d the density, N Avogadro's number, k Boltzmann's constant, T the absolute temperature, α the polarizability. This result was dis-

puted by the old quantum theory, but has been confirmed by the new wave mechanics.

A molecule is dipolar if the moments of its charges about the coördinate axes, $\Sigma e_i x_i$, $\Sigma e_i y_i$, $\Sigma e_i z_i$ do not all vanish. If the moments vanish, but what might be called the inertial moments, $\Sigma e_i x_i^2$ etc. do not, the molecule has a quadrupole: the effect of this may, however, be neglected when there is an appreciable dipole.

The potential of a dipole whose center is at O is, at a point P, $\dfrac{\mu \cos \theta}{r^2}$, where $r = OP$, and θ is the angle between OP and the dipole axis. The mutual potential energy of two dipoles, μ, μ' is

$$\frac{\mu\,\mu'}{r^3}\,(\sin\theta \cdot \sin\theta' - 2\cos\theta\cos\theta')$$

where the line joining their centers O, O' makes angles θ and θ' with the axes of the dipoles, and $r = OO'$.

The dipole theory of adsorption

There have been various attempts to interpret adsorption in terms of polarization. In general, the conditions are so complex that only a partial treatment has been adopted, applicable to special cases.

At first sight the adsorption of a polar molecule such as water seems readily explained by the presence in the water of an electric doublet. The molecules would stick to the surface just as would small magnetic doublets to the surface of cast iron. The attraction is caused by the charges induced on the surface, and these can be replaced, as Kelvin showed, by the mirror image of the inducing charges. While all metals would exert the same attraction, dielectrics would differ, as in this case the induced dipole is $\mu \left(\dfrac{\epsilon - 1}{\epsilon + 1} \right)^{\frac{1}{2}}$, where ϵ is the dielectric constant of the adsorbent. However, this treatment uses the theory of images in the atomic realm without justification, and it leaves out of account altogether the effect of the surface field of the adsorbent. Thus mirror image forces play only a partial rôle in adsorption.

Landé and Lorenz (38) write the energy of a permanent dipole μ in the field of its mirror image in the form

$$\frac{(1 + \cos^2 \beta)}{8 \, \delta^3}$$

where the dipole is distant δ from the surface, and makes an angle β with the surface normal. The number of molecules whose angles with the surface normal are between β and $\beta + d\beta$ is therefore

$$x_\infty \, e^{\frac{\mu^2 (1 + \cos^2 \beta)}{8 \, \delta^3 kT}} \cdot 2 \pi \sin \beta \, d\beta$$

Averaging over all angles, the number of molecules adsorbed per sq. cm. is found to be

$$x_\infty \frac{\delta_0}{6} \cdot \frac{e^{2 y^0}}{y_0^2}$$

where x_∞ is the concentration of the bulk gas, δ_0 is the radius of a molecule, and

$$y_0 = \frac{\mu^2}{8 \, \delta_0^3 \, kT}$$

However, when x_∞ corresponds to an atmospheric pressure at 0°C. and $\mu = 10^{-18}$, only a small fraction of the surface can be covered on this basis.

When the effect of a surface field E on the permanent dipoles (without electron polarization) is included, so that the energy of a dipole is

$$\frac{\mu^2}{\delta^2} (1 + \cos^2 \beta) + \mu \, E \cos \beta$$

Blüh and Stark (39) find that the adsorption without E must be multiplied by a factor $e^{\frac{\mu E}{kT}}$. When μ is 10^{-18} this factor is given for various values of E (E.S.U.)

E......	10^4	10^5	2×10^5	2.5×10^5	3×10^5	5×10^5	8×10^5	3×10^6
$e^{\frac{\mu E}{kT}}$....	.01	.3	1	2	3	9	23	525

They find that for an ionic lattice with a lattice constant 2
A. U. the field at a distance 1 A. U. from the surface is 4.3×10^6
E.S.U., sufficient to account for adsorption. A similar calcula-
tion has been made by Lennard-Jones (40) for the 100 plane of
NaCl: the van der Waals, as well as the ionic, field was studied.

Zahn (41) measured the dielectric constant ϵ of water at various
temperatures and pressures. He found that $\epsilon - 1$ plotted
against the pressure gave two intersecting straight lines. This is
inconsistent with Jona's (42) interpretation of the anomalous
behavior of water vapor as due to association. Zahn assumes
that the dipoles condense on the electrodes, and finds that on the
basis of mirror image forces

$$= c_\infty \left(1 + \frac{y_0}{3} + \frac{y_0^2}{10} + \frac{y_0^3}{4^6} + \frac{y_0^4}{4^{16}} \right)$$

where c is the concentration at a distance δ_0 from the electrode
surface, and y_0 is as above. At the pressure at which deviations
from normality first occur

$$\frac{c}{c_\infty} = \frac{\text{saturated pressure}}{\text{equilibrium pressure of film}}$$

$$= \frac{20}{6.2} \text{ at } 23.3°C.$$

This gives y_0 the value 2.5 and thence δ_0 is found to be 2.2 A. U.,
which agrees well with the radius of a water molecule.

The above theory of the action of mirrow image forces is
inadequate in two respects. The electron polarization of the
adsorbed molecules is neglected, as is the interaction between the
various adsorbed molecules and their mirror images. Bradley
(43) assumes the first dipole, the one next to the surface, to be
strongly held and completely oriented by the surface forces, and
studies the effect of building up successive layers. The surface
forces are supposed not to extend beyond the first dipole, whose
energy is not considered. For simplicity this three-dimensional
problem is replaced by one-dimensional study of an adsorption
chain perpendicular to the surface, which is strictly the case
already considered. Consider n dipoles of diameter a to form

the chain; let the force on the pth dipole be F_p; let μ_s be the moment, perpendicular to the surface, of the sth dipole. Then

$$F_p = \sum_{s=1}^{s=p-1} \left[\frac{2\,\mu_s}{(p-s)^3\,a^3} + \frac{2\,\mu_s}{(p+s-1)^3\,a^3} \right] +$$

$$\sum_{s=p+1}^{s=n} \left[\frac{2\,\mu_s}{(s-p)^3\,a^3} + \frac{2\,\mu_s}{(p+s-1)^3\,a^3} \right] + \frac{2\,\mu_p}{(2\,p-1)^3\,a^3}$$

This equation merely gives the force on the pth dipole due to dipoles on either side, and due to the mirror images of the sequence of dipoles. Also

$$\mu_s = \mu\, L\left(\frac{\mu\, F_s}{kT}\right) + \alpha\, F_s$$

where

$$L(x) = \coth x - \frac{1}{x} = 1 - \frac{1}{x} \text{ for } x \gg 1$$

Since $\dfrac{\mu E}{kT} > 1$ for a molecule such as water at room temperature, all the μ's in the equation for F_p can be replaced by F's. To a first approximation these n equations are linear in F, writing

$$1 - \frac{kT}{\mu\,F} = 1$$

and the various F's can be found in the form of determinants. The second approximation is then obtained by inserting the values of F so found in the terms $1 - \dfrac{kT}{\mu F}$ and resolving for F. In this way F is calculated, and the energy of the pth dipole is

$$- \mu\, F_p\, L\left(\frac{\mu\, F_p}{kT}\right) - \tfrac{1}{2}\,\alpha\, F_p{}^2 \qquad \frac{2\,\lambda}{(n-1)\,a^{n-1}}$$

the last term representing the repulsive energy. The evaluation of the latter is uncertain. It may be computed on the assump-

tion of a neon-like sheath for the pseudo-atom water, and is
relatively small. The calculation is laborious, and was performed
only for layers of two and three molecules. The difference
between the total energies of chains of three and two molecules
gives the energy required to remove a molecule from a chain of
three molecules, and this is the latent heat of evaporation, per
molecule, of the film. In the case of water at 25°C. we find for a
layer of three molecules $F_3 = 1.61 \times 10^5$, $F_2 = 2.85 \times 10^5$,
$F_1 = 3.13 \times 10^5$. And for a layer of two molecules $F_2 = 1.62 \times$
10^5, $F_1 = 2.69 \times 10^5$. This gives 8600 calories for the latent
heat of evaporation per gram-molecule of a film of three mole-
cules, agreeing well with Lehner's (44) figures for

$$- R\, d_n\, \frac{(\log p_n)}{\left(\dfrac{1}{T}\right)} - r$$

n as above, viz., at 25°C. $n = 5, L = 9100; n = 10, L = 10530$.

By a calculation in some respects similar to the above, in
that adsorption is viewed as the building up of successive layers,
rather than the establishment of a concentration gradient up to
the surface, Boer and Zwikker (45) have explained the peculiar
adsorption isotherm of a polarizable molecule (without permanent
dipole) on an ionic lattice. The amount adsorbed at first in-
creases quickly with p, the pressure, then more slowly, and finally
near saturation, there is again a rapid rise; in no case does n
remain constant as p increases.

Let p be the moment induced in a molecule in the nth layer
from the surface, b the distance between adjacent molecules in a
layer, c the distance between adjacent layers. The energy ϕ_1 of
the first layer is

$$- A\, p_1 + \frac{B\, p_1{}^2}{b^3} - \frac{C}{c^3}\, p_1 p_2 - \frac{D}{c^3}\, p_1 p_3 \dots\dots$$

where A, B, C etc. are summation constants for the action of the
ionic lattice, the neighboring dipoles in the first layer, those in
the second layer, and so on. Similarly

$$\phi_k = \frac{p^2 k}{2\,\alpha} + B\, \frac{p^2 k}{b^3} - C\left[\frac{p_{k-1} \cdot p_k}{c^3} + \frac{p_k\, p_{k+1}}{c^3}\right] - D\left[\frac{p_{k-2}\, p_k}{c^3} + \frac{p_k\, p_{k+2}}{c^3}\right] + \dots.$$

Here no account is taken of mirror images. The total energy per sq. cm. is

$$\frac{\phi}{N} = -A\,p_1 + \left(\frac{B}{b^3} + \frac{1}{2\,\alpha}\right)\Sigma\,p_k{}^2 - \frac{C}{c^3}\Sigma\,p_k\,p_{k+1} - \frac{D}{c^3}\Sigma\,p_k\,p_{k+2}\dots.$$

Since the variables are $p_1 \dots p_n$ for equilibrium $\dfrac{d\phi}{dp_k} = 0$. These differentiations give the series of equations

$$\beta\,p_1 + \gamma\,p_2 + \delta\,p_3 + \dots\dots\dots = A$$

$$\gamma\,p_1 + \beta\,p_2 + \gamma\,p_3 + \delta\,p_4 + \dots = 0$$

$$\gamma\,p_{n-1} + \beta\,p_n\dots\dots\dots\dots = 0$$

where β, γ, δ, are constants. Solution of these gives ϕ_n in the approximate form

$$\frac{-A^2}{2\,\beta} \cdot \left(\frac{K}{1-K^2}\right)^{2n-2}$$

where K is a constant. Knowing ϕ_n, π, the pressure of gas in equilibrium with the adsorbed film, can be found in terms of unknown constants (as an approximation) giving the isotherm

$$\log\frac{\pi}{K_3\,\pi_0} = K_2\,K_1{}^n$$

where π_0 is the vapor pressure of the liquid, and K_1, K_2, K_3, are constants. This gives a curve exactly of the form found experimentally by Boer (46) for the adsorption of iodine on calcium fluoride.

A similar calculation has been made by Herzfeld (47), who considers the interaction between pairs of adjacent molecules in the layer next to the surface, with a view to explaining the change in the heat of adsorption with the amount adsorbed. According as the two adjacent polarizable molecules are adsorbed on ions of the same, or of opposite sign, the heat of adsorption is lowered

or raised as the number of pairs increases. Calculation shows that repulsion between adjacent molecules of an agglomerate could not change the heat of adsorption for a salt, and might lower it by about 10 per cent for a metal: attraction could increase the heat by about 10 per cent for salts, and by about 40 per cent for metals.

In view of the complexity of the polarization phenomena of adsorbed films one would not expect the simple relation between dielectric constant and amount adsorbed found by Illin (48). His work has been criticized by Hückel (49), and by Cassel (50).

The effect of adsorption on electric surface properties

No extensive study has been made of the effect on adsorption of varying the surface field, as high enough fields are not under our control. Loeb (51) found that water would condense first on a negative electrode, in a vessel containing both electrodes, which is in line with the condensation of water vapor on the negative ions first in the Wilson expansion chamber. Evidently water has an asymmetric dipole. However, the effect of the surface film on various electric properties has been studied, notably the effect on the polarization of reflected light, photo-electric and thermionic emission, and the diffraction of electrons.

When light incident at the polarizing angle is reflected from a plane surface, the reflected beam is not plane, but elliptically, polarized. Drude explained this by assuming a gradually changing transition region between the two media. If ρ is the ellipticity, ϵ_1, ϵ_2, ϵ the dielectric constants of the first, the second and the transition media, then

$$\rho = \frac{\pi}{\lambda} \cdot \frac{(\epsilon + \epsilon_2)^{\frac{1}{2}}}{\epsilon_1 - \epsilon_2} \int \frac{(\epsilon - \epsilon_1)(\epsilon - \epsilon_2)}{\epsilon} \cdot dz$$

where z is the position in the transition region. This sets a lower limit to the thickness of the adsorbed layer, not very different from the actual value, equal to

$$\frac{\lambda \rho}{\pi (1 + n_1^2)^{\frac{1}{2}}} \cdot \frac{n_1 + 1}{n_1 - 1}$$

where n_1 is the refractive index of medium 2 with respect to medium 1. Frazer (52) and Silverman (53) have used this method to study the formation of films, Frazer using water and methyl alcohol on glass, Silverman methyl alcohol on rock salt. The thickness of the layer could be determined to .5 A. U. Silverman found that between 1×10^{-5} and 1×10^{-4} cm. the first layer was built up and that there was no further adsorption until the pressure reached 2 to 3 cm. From this pressure until about 10 cm. the second layer was attached. The first layer was about 4.9 A. U. thick, the second 4.3 A. U. These pressures are what we should expect if the first molecule is held by the surface force of the rock salt, and the second by cohesive forces between methyl alcohol molecules.

Surface films affect thermionic and photo-electric emission by providing at the surface an additional potential step, which may be positive or negative. If x is the number of atoms per sq. cm., ΔV, the potential increment, is equal to $4 \pi \times \mu$. If the fraction covered is θ the thermionic current is therefore of the form

$$i_\theta = i A T^2 e^{\frac{\phi + \alpha_1 \theta}{kT}}$$

where α_1 is positive or negative. Thus the effect of the surface film is very great, a monatomic layer of thorium or tungsten at 1500°C. increasing the emission by a factor of 10^5. A wave mechanics study of the effect of the modification of the surface potential hump has been made by Georgeson (54), following Nordheim (55). He finds that the A of Richardson's formula is of the form

$$B \, l^{\frac{1}{2}} \, (\delta \chi)^{\frac{1}{2}} \, e^{- .7 \, l \, (\delta \chi)^{\frac{1}{2}}}$$

where B is a constant, l is the thickness of the layer, $\delta \chi$ is the potential drop in the layer. In this way he deduces a value 5.3 A. U. for the thickness of a layer of thorium on tungsten, a reasonable result.

The study of the interference beams formed by reflection of electrons offers an interesting method of investigating surfaces. This method is especially useful if slow electrons are employed,

for these penetrate only a short distance into the interior. As an example, Rupp (56) found that with hydrogen on a nickel surface new interference maxima appear, in addition to those for clean nickel. On standing for two days only the latter change, becoming smaller. This is ascribed to the penetration of the gas into the nickel lattice, where it is irregularly arranged, in contrast to the gas on the surface. On heating, the new hydrogen maxima disappear, leaving those for nickel lower than for the clean surface.

Germer's work (18) has already been referred to (Section I). G. P. Thompson has used fast electrons to investigate surfaces.

Gas films do not influence the diffraction. Reflection measurements may be made photographically, and there is in general a diffuse diffraction, on which may be superimposed a system of spots, due to a single surface crystal, or concentric rings, due to a polycrystalline surface. Polished copper gives the diffuse background, but on standing in air faint rings appear, whose intensity and clearness are greatly increased by heating the copper. The rings correspond to a cuprous oxide lattice. Similarly, passive iron, whose original polish had been only slightly dimmed, gave good rings corresponding to ferric oxide. The passivity had, however, probably been lost during the test, and when special care was taken no change could be observed from polished iron.

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STRUCTURE OF DIVALENT CARBON COMPOUNDS

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The divalent carbon atom is recognized as occurring in three classes of compounds—carbon monoxide, the isocyanides or carbylamines, and the fulminates. The last of these needs no separate treatment; it was proved by Nef that the fulminates have the same relation to the isocyanides that the normal cyanates have to the nitriles,

| Nitriles | R—CN | Normal Cyanates | R—O—CN |
| Isocyanides | R—NC | Fulminates | R—O—NC |

and any conclusions as to the structure of the $-NC$ group in the isocyanides apply equally to the fulminates. For carbon monoxide and the isocyanides three types of formulas have been proposed:

$$O\equiv C \qquad\qquad O=C \qquad\qquad \left.\begin{array}{c} O\overset{-}{\underset{}{\rightleftharpoons}}C \\ \overset{+}{O}\equiv\overset{-}{C} \end{array}\right\}$$

$$R-N\equiv C \qquad\qquad R-N=C \qquad\qquad \left.\begin{array}{c} R-N\overset{-}{\rightleftharpoons}C \\ R-\overset{+}{N}\equiv\overset{-}{C} \end{array}\right\}$$

$$(1) \qquad\qquad\qquad (2) \qquad\qquad\qquad (3)$$

The first of these has long been discarded on stereochemical grounds, since the tetrahedral atomic model does not admit of a quadruple link; it is also inconsistent with the modern view that the covalence of nitrogen cannot exceed four. It was replaced more than thirty years ago by the second type, mainly through

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the work of Nef (1), which will be discussed later. The third, which can be written in two ways both meaning the same thing, namely that an electron is transferred from the oxygen or nitrogen to the carbon and that six electrons are shared between the two atoms,

$$:O \vdots C: \qquad R:N \vdots C:$$

was suggested by Langmuir (2), and has been accepted by G. N. Lewis (3), Lowry, Sugden (4), and others; but it has not been definitely established, nor have its implications been discussed in detail, so that it has not displaced the Nef formula in general use, especially among organic chemists, who are those mainly interested in this question.

In this paper is discussed further evidence that seems to put the correctness of the Langmuir formula beyond doubt.

The most striking is that of the dipole moments. We know now that in the covalent link the two electrons are not as a rule shared equally between the two atoms, so that the system forms an electrical dipole, more positive towards one end and more negative towards the other. The moment of a dipole is the product of the electric charge into the distance of separation; the sum of the moments in a molecule can be determined experimentally, and the deduction of the moments of individual links, though it is only approximate, is near enough to give us valuable information. The moments are expressed in terms of the unit 10^{-18} E.S.U.

Carbon monoxide has been found to be almost non-polar; its moment[2] is 0.12. Now a single C—O link has a moment of 0.7, and the double C=O link in aldehydes and ketones has one of about 2.3, the oxygen being at the negative end. Hence· if carbon monoxide had the structure C=O it should have a moment of 2 or 3. The fact that it is almost non-polar shows that there is a counterbalancing moment in the molecule. This is provided

[2] Moments quoted in this paper without authority are taken from Debye, Polare Molekeln, Leipzig, 1929, including the supplementary list issued in January, 1930.

by the transferred electron of the Langmuir formula. We can even calculate approximately what its effect should be. The distance between the atomic centers in carbon monoxide has been shown to be 1.14 A.U. (5). If we may assume the electron to be transferred through this distance, it will produce a moment of $1.14 \times 4.77 = 5.44$ units. This differs from the moment of the triple $C \equiv O$ link, and we may assume exceeds it, by 0.12. Thus it appears that the moment of the triply linked $C \equiv O$ without the effect of the transferred electron would be 5.3. It may be pointed out that this makes the ratio of the moments of $C \equiv O$ and C—O (5.3 ÷ 0.7 = 7.6) almost the same as that of the triple and single links of carbon to nitrogen (3.8 ÷ 0.4 = 9.5). At any rate the effect is of the right order, and in the right direction.

Coming to the nitriles and isocyanides, we know that the nitrile group —$C \equiv N$ has a large moment (benzonitrile, 3.9), and we might assume that the nitrogen would be at the negative end; but to make certain, this was determined by the ingenious method of J. W. Williams of balancing the group against another in the *para* position in the benzene nucleus. *p*-Nitrobenzonitrile was found to have a moment of 0.7 (6), showing that the two groups oppose one another. In —NO_2 the negative end of the dipole must be remote from the ring; hence this is so with the nitrile group as well.

The magnitude and direction of the moment of the isocyanide group were determined in the same way (6). *p*-Tolyl isocyanide was found to have a moment of 3.98, and *p*-chlorophenyl iso-cyanide one of 2.07. Now toluene has a moment of 0.43, and the positive end is away from the ring: chlorobenzene has a moment of 1.56 in the opposite direction (see table 1). It is evident that the moment of —NC is strengthened by methyl and weakened by chlorine; the carbon must therefore be negative to the nitrogen. The magnitude of the moment is from the tolyl compound $3.98 - 0.43 = 3.55$, and from the chloro compound $2.07 + 1.55 = 3.62$. The moment of phenyl isocyanide itself has since been determined (7), and was found to be 3.49, which is in satisfactory agreement with our results.

If the isocyanide had the formula C_6H_5—N=C its moment
would be essentially the resultant of two, $\overrightarrow{C-N}$ and $\overleftarrow{N=C}$; in
both the nitrogen is negative, but more strongly in the double

<div align="center">

TABLE 1

Dipole moments ($\times 10^{18}$ *E.S.U.*)

</div>

link. The value for C—N is 0.4, and that for N=C would pre-
sumably be from 1 to 1.5. The whole group should thus have a
moment of about 1, of which the positive end would be remote
from the ring. The observed facts, that the moment is about

3.5, and still more that it is in the opposite direction, are incompatible with this structure, but are explained by the coördinate formula as due to the transferred electron, which, as in carbon monoxide, but much more strongly, overcomes the moment due to the inequality of sharing of the six electrons of the triple link. Here too we can make an approximate calculation.   In the group $>$C—N$\equiv$C we have three moments concerned: (*1*) that of $\overset{\longleftrightarrow}{\text{C—N}}$ (0.4); (*2*) that of six shared electrons in $\overset{\longleftarrow}{\text{N}\equiv\text{C}}$, which may be assumed to be 3.8, as in the nitriles; and (*3*) that due to the transferred electron in $\overset{\longrightarrow}{\text{N}\equiv\text{C}}$, which is 4.77 $\times$ *d*, where *d* is the distance of transference.   Since the total moment is found to be 3.5, we have

$$3.5 = 4.77 \times d + 0.4 - \text{about } 3.8$$

whence

$$4.77 \times d = 6.9$$

or

$$d = 1.4 \text{ A.U.}$$

The distance between the atoms in the —NC group is unknown; but it may be taken to be about the same as that between the triply linked atoms of carbon in acetylene (1.2 A.U.) or of nitrogen in nitrogen gas (1.1 or 1.2 A.U.), and so to be 1.2 A.U.   This again agrees well with the value deduced from the moments.

These calculations have an interest outside this particular group of compounds.   There is a tendency to assume that the idea of the transferred electron in the coördinate link should not be taken too seriously; and it is important to realize that when the idea can be tested quantitatively by dipole measurements, it is found to be in accord with experiment.

The coördinated formulas for these compounds are further supported by the heats of formation of the links from their atoms, as calculated by the method of Grimm and Wolff (8). The relevant values are given in table 2, expressed in kilogram-calories per gram-molecule.   They have been recalculated in the light of recent determinations of the heats of dissociation of oxygen and nitrogen.

It will be seen that in each case the heat of formation is approximately proportional to the multiplicity of the link, and that both carbon monoxide and the isocyanides behave as triply linked compounds.

The parachors are also in agreement with these formulas. The meaning of the parachor is still to some extent a mystery, but it is a definite additive property, particularly suitable for detecting multiple linkage. The parachors of the isocyanides were measured simultaneously at Oxford (6) and by Lindemann

TABLE 2

Heats of formation of links

LINK	COMPOUND	HEAT OF FORMATION	
		Abs.	Rel.
		kg. cal per gram-molecule	*kg.-cal. per gram-molecule*
C—O	Alcohols	73.0	1
	Ethers	73.5	1
C⁝=O	Aldehydes	163	2.2
	Ketones	163	2.2
	Carbon dioxide	180.7	2.5
C≝O	Carbon monoxide	237	3.2
C—N	Monamines	58.5	1
C=N	Isocyanates	108 9	1.9
C≡N	Nitriles	185.4	3.2
	Isocyanides	179 7	3.1

(9) in Germany. The results for carbon monoxide (10) and the isocyanides are given in table 3, along with the values calculated for the Nef and Langmuir formulas. In calculating the value for the Nef formula it is assumed that the carbon exerts its normal effect on the parachor. Although this is not certain, all analogy shows that the reduction which that formula requires, of the valence octet to a sextet, and of the covalence from four to two, if it had any effect would diminish the parachor, so that the value given in the table is a maximum. With carbon monoxide the observed value is at least much nearer to that requiied for the

second formula, and with the isocyanides the agreement with the latter is reasonably good. .

The volatility is also in agreement with this theory, although that property depends directly on the dipole moments, and not on the formula assigned to the group. The presence of a coördinate link normally depresses the volatility of a compound, but, except where association occurs, this is only because it increases the dipole moment. In the compounds we are considering, the coördination does not increase the moment; in carbon monoxide

TABLE 3

Parachors

CARBON MONOXIDE	P (OBSERVED)	P (CALCULATED)	
		$C=O$	$C\equiv O$
	61.6	48.0	69.6

ISOCYANIDES R—NC	P_{NC} (observed)		P_{NC} (calculated)	
	Lindemann and Wiegrebe	Hammick, Now, Sidgwick and Sutton	$R-N=C$	$R-N\equiv C$
R = Methyl........	66.0			
Ethyl........		69		
Phenyl................	65.2			
o-Tolyl............	63.9			
p-Tolyl......................	66.5	66	40.5	62.3
o-Anisyl....................	65.1			
p-Anisyl....................	65.5	66		
Mean 65.5				

it almost destroys it. The close agreement in critical data between carbon monoxide and nitrogen to which Langmuir (2) drew attention, was inexplicable as long as it was supposed that the former gas was highly polar, but it is explained by its actual polarity being so small. The cyanides and the isocyanides having nearly equal moments should be about equally volatile, and as a fact the cyanides boil about 20° higher than their isomers, which corresponds to a difference in the heats of evaporation of only about 5 per cent.

The coördinate structure with the triple link is thus supported by the physical data.   It is also in accordance with the chemical properties.   The main point on which Nef based his formula was the evidence he obtained that in the isocyanides (and in the fulminates) the unsaturated character is confined to the carbon, and does not extend, as it does in the nitriles, to the nitrogen, their addition compounds (for example with chlorine, oxygen, and ethyl hypochlorite) being always of the type R—N=CX$_2$.   This behavior is equally to be expected from the Langmuir formula. In a molecule R—N≡C the nitrogen has a fully shared octet, so that, like carbon in the tetrachloride, it cannot coördinate either as donor or as acceptor.   The carbon has a lone pair of electrons, and from its strong tendency to assume the 4-covalent state, it is to be expected that these will readily react.   Further, the coördinate link with the nitrogen is easily broken, as such links always are, by the return of the two electrons to the exclusive control of the nitrogen, and the carbon can then act as acceptor.   Hence the reactivity of the group resides in the carbon alone.   In the nitriles, on the other hand, the nitrogen has a negative charge and a lone pair, so that it can act as a donor, as it does in many complexes formed by nitriles, but as its covalence is limited to four, it cannot use these two electrons to form two new covalences; any further reaction involves the rupture of a link between the nitrogen and the carbon, and hence addition to both of these atoms.

Further support is given to this view by the behavior of carbon monoxide and the isocyanides in complex formation.   The complexes formed by carbon monoxide, especially the carbonyls, are not fully understood, but it is clear (1) that the CO groups are separately attached to the central atom, since they always come away separately; (2) that they invariably act as donors and not as acceptors, since they always attach themselves to atoms which can take up other donors like ammonia; and (3) from the composition of the "mixed" carbonyl compounds such as [Pt(NH$_3$)$_2$(CO)$_2$]Cl$_2$, that each CO occupies one coördination place, that is, provides one pair of electrons, like a molecule of water or ammonia.   The complexes formed by the isocyanides, for example

[Pt(CH$_3$·NC)$_2$Cl$_2$] show that they behave in the same way. This is in complete agreement with the coördinate structure, in which the carbon has a lone pair, and, to judge from the stability of the fully shared octet in carbon, must be very willing to share it. If, however, the Nef formula were correct and the carbon had only six valence electrons, it is incredible that it should never complete its octet by acting as acceptor.

The recognition of these structures removes an apparent anomaly in the behavior of carbon as compared with its allies in the fourth group of the Periodic Table. The "inertness" of a pair of valence electrons, changing the valence by two units, is common among the elements of the B subgroups, but in any group it is always found to be most marked in the heaviest members. Thus its intensity is in the order I > Br (Cl, F); Te > Se (S, O); Bi > Sb > As (P, N). The elements in brackets are those which show no sign of this inertness. In the fourth group this order is very clearly defined; the inertness is most marked in lead, where the only stable ion is divalent, less in tin (Sn^{++}), faint in germanium, and practically absent in silicon. It would be against all analogy that it should then reappear in force in the lightest member, carbon.

The whole of organic chemistry shows that the general characteristic of carbon is to be stable only in the normal 4-covalent state, and to refuse to form either coördinate links or electrovalences. But it is evident that in these divalent compounds we have an exception to this rule, and the carbon is acting as acceptor. This suggests that the same thing may happen elsewhere, and may have been overlooked; that, for example, in some compounds in which the grouping C=X has been assumed, this should be written C←X. The presence of the coördinate link would be detected through the properties of the compound, and through the difference involved in the valence group of the atom X. An instance of this, where X is a sulfur atom, has been suggested by Ingold and Jessop (11), but perhaps the most interesting example is the following one. Kuhn has shown (12) that the optically active form of

$$\begin{array}{ccc} C_2H_5 & & H \\ & \diagdown \; \diagup & \\ & C & \\ & \diagup \; \diagdown & \\ CH_3 & & NO_2 \end{array}$$

retains a considerable part of its activity after solution in alkali. This remarkable result has been confirmed by Shriner and Young (13). Now in the alkaline derivatives the metallic atom is undoubtedly ionized, and it has always been assumed, since the work of Hantzsch, that the ion had the structure

$$\begin{array}{c} C_2H_5 \\ \diagdown \\ \quad\quad C{=}NO_2{}^- \\ \diagup \\ CH_3 \end{array}$$

But this is symmetrical, and if it were formed, solution in alkali must destroy the optical activity. Kuhn, however, points out that if we assume that the nitrogen is joined to the carbon in the ion by a coördinate link,

$$\begin{array}{c} C_2H_5 \\ \diagdown \\ \quad\quad C \leftarrow NO_2{}^- \\ \diagup \\ CH_3 \end{array}$$

the ion is not symmetrical, and the persistence of the activity is explained: the central carbon atom has the same possibility of activity as the 3-covalent sulfur atom in the compounds of Phillips and Kenyon (14). If the optical activity found by Levene and by W. A. Noyes (15) in the aliphatic diazo compounds, which has recently been confirmed by Lindemann (16), is really due to these compounds and not to an impurity, we must assign to them a similar structure

$$\begin{array}{c} A \\ \diagdown \\ \quad\quad C \leftarrow N{\equiv}N \\ \diagup \\ B \end{array}$$

Another group of compounds was supposed by Nef to contain divalent carbon: he maintained that acetylene and its derivatives could occur in tautomeric forms

$$R—C\equiv C—R \rightleftharpoons R_2C=C.$$

These cannot be explained in the same way; the central carbon atom of the second formula has a fully shared octet and no lone pair, so that it cannot coördinate. If this formula is correct the terminal carbon here has only a valency sextet. These views of Nef are however doubtful (17). Recent work on the band spectrum of acetylene has shown that at least the greater part consists of symmetrical linear molecules $H—C\equiv C—H$. It should be easy to settle the question by measuring the dipole moment of such a compound as diiodoacetylene. $I—C\equiv C—I$ would be nonpolar, and $I_2C=C$ would be highly polar. The existence of the supposed acetal of carbon monoxide, $C(O\cdot C_2H_5)_2$ (18), seems from the recent work of Arbusow (19) to be very doubtful.

We may still call the isocyanides, the fulminates, and carbon monoxide compounds of divalent carbon. The only satisfactory definition of the numerical valency of a combined atom is that suggested by Grimm and Sommerfeld, that it is the difference between the number of unshared electrons that the atom has in the uncombined state (atomic number) and the number that it has when combined. In these compounds the carbon has a valence group of two unshared and six shared electrons; it has shared two of its valence electrons, and in this sense its valence is two, but its covalence, which according to the Nef theory was two in all three classes of compounds, now appears as three.

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THE HEATS OF DILUTION OF STRONG ELECTROLYTES

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INTRODUCTION

With the appearance of Debye and Hückel's theory of strong electrolytes (1) increased interest and importance became attached to the values of the heats of dilution of these substances as one means for testing and widening the applicability of the theory. Gross and Halpern (2) and Bjerrum (3) treated the theoretical derivation more strictly and the latter derived the following expression for the integral heat of dilution of a strong electrolyte as a limiting expression for extreme dilutions, the ions being regarded as point charges.

$$V_c = -\frac{kT\kappa^3}{8\pi}\left(1 + \frac{T}{D}\frac{dD}{dT}\right) \text{ calories per mole of salt} \qquad (1)$$

$$\kappa^2 = \frac{4\pi e^2}{DkT}\Sigma n_i z_i^2$$

e = elementary quantum of electricity,
D = dielectric constant of the medium,
k = gas constant per molecule,
T = absolute temperature,
n_i = number per cubic centimeter, and
z_i = valence of an ion of the jth kind.

A comparison with the then available data obtained in dilute solutions—measurements of Richards and Rowe (4) on uni-univalent salts which extended to a minimum concentration of 1 mole salt plus 400 moles water—showed that the experimental values did not agree at all with equation 1; the measured heats of dilution were in a number of cases not even of the predicted positive sign. Bjerrum (5) later attempted to explain these anomalies by use of the parameter 'a' (an average value of the closest distance of approach of two ions in the solution) introduced by Debye and Hückel, suggesting that 'a' varied with T; also that the effective dielectric constant decreased in the immediate neighborhood of the ions. At about the same time measurements were made in more dilute solutions by Nernst and Orthman (6) which still gave negative heats of dilution for several of the salts measured, even at the lowest concentration. In 1927 it was shown independently by the same authors (7) and by Lange and Messner (8) that at sufficiently great dilution the heat of dilution was positive for all salts measured and that for 1–1 salts the observed values agreed fairly well with each other, as demanded by the limiting form of the Debye-Hückel theory. A more exact treatment of the fundamental theory of Debye and Hückel by Gronwall, LaMer and Sandved (9) was applied by Lange and Meixner (10) to a new calculation of theoretical integral heats of dilution, giving the following expression

$$V_e = -\frac{N}{n} \sum_{j=1}^{s} n_j \frac{z_j e}{D a_j} \left[\psi_j - \frac{z_j e}{D a_j} \right] \left[1 + \frac{T}{D} \frac{dD}{dT} \right] \text{ergs per mole of salt} \quad (2)$$

The integral heat of dilution is accordingly equal to the ionic electrical potential energy at the concentration c (expressed in moles of salt per liter of solution) less that at infinite dilution multiplied by the factor $\left(1 + \frac{T}{D} \frac{dD}{dT} \right)$ which is characteristic of the solvent alone. It is important to observe that the following assumptions are made use of in the derivation of this expression: (1) strong electrolytes are completely dissociated at all concentrations; (2) the ions are rigid charged spheres with average least distances of approach 'a'; (3) only Coulomb forces act between the ions (11);

(*4*) the influence of the solvent is represented by its dielectric constant alone, the value of D (12) being independent of the number and nature of the ions present, $dD/dc = 0$; (*5*) the distribution of the ions in the solution is determined by their thermal motion and the Coulomb forces acting among them, the only individual factor being the value of the parameter 'a'; the combined use of Boltzmann's and Poisson's equations gives an accurate picture of this iónic distribution (13); (*6*) in developing equation 2 the additional assumption $d\,'a'/dT = 0$ is necessary.[1]

For aqueous solutions at room temperature equation 2 also demands that V_c should always be positive,[2] i.e., heat should be evolved by the dilution. For extreme dilutions the limiting law retains its validity and V_e becomes proportional to $\sqrt{c}$ ($V_e = A\sqrt{c}$).

To evaluate V_c, experimental values for D and dD/dT must be substituted in equation 2. In table 1 are compared the most important measurements reported in the literature to date, together with values for $\left(1 + \dfrac{T}{D}\dfrac{dD}{dT}\right)$ and A for 1–1 salts calculated from these measurements.

It is seen that the values of $\left(1 + \dfrac{T}{D}\dfrac{dD}{dT}\right)$ and A are very sensitive to small changes in dD/dT. Certainly $d\dot{D}/dT$ for water is not known with sufficient accuracy (15) to permit a comparison of measured integral heats of dilution with those calculated from equation 2. It would be necessary to know dD/dT with an accuracy of ± 0.5 per cent, an accuracy which has probably not yet been reached with the experimental means available today. Perhaps the reason for the lack of agreement lies in some systematic errors peculiar to the various methods employed (16).

The correct values probably lie between those of Drude (14b) and those of Wyman (14i) given in table 1, but for the present a direct numerical comparison does not seem worth while. The theory can still be tested however with respect to the predicted

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[1] Since this article was submitted, Scatchard has pointed out (J. Am. Chem. Soc. **53**, 2037 (1931)) the omission of a term in $d\ln V/d\ln T$ from equation 2, which would reduce the slope A in the limiting law by about 7 per cent for water.

[2] In the notation of Lewis and Randall, "Thermodynamics," p. 88, $V_e = -n_1\bar{L}_1 - \bar{L}_2$, where n_1 moles of water contain one mole of salt.

TABLE 1

Values of D, dD/dT, $\left(1 + \dfrac{T}{D}\dfrac{dD}{dT}\right)$ and A for 1-1 salts

AUTHOR	REFERENCE	12.5°C.				25°C.			
		D	$\dfrac{dD}{dT}$	$\left(1 + \dfrac{T}{D}\dfrac{dD}{dT}\right)$	A	D	$\dfrac{dD}{dT}$	$\left(1 + \dfrac{T}{D}\dfrac{dD}{dT}\right)$	A^*
Drude (graphical)	(14a)	82 77	-0.368	-0.269	351	78 26	-0.349	-0.329	457
Drude (formula)	(14b)	83.34	-0.379	-0.299	386	78 77	-0.353	-0.337	464
Kockel (graphical), confirmed by Devoto	(14c, d)	82 81	$-0\ 404$	$-0\ 393$	513	77.84	-0.385	-0.474	664
Kockel (formula of Adams)	(14e)	82 92	-0.436	-0.500	651	77.76	-0.391	-0.500	702
International Critical Tables	(14f)	83	-0.4	-0.376	489	78	$-0\ 4$	-0.528	738
Cuthbertson and Maass (graphical)	(14g)	79 42	-0.368	$-0\ 323$	449	75.40	$-0\ 289$	-0.142	209
Drake, Pierce and Dow (formula)	(14h)	83.29	-0.393	$-0\ 346$	448	78.57	-0.362	-0.374	517
Wyman (formula)	(14i)	83.16	$-0\ 379$	$-0\ 300$	389	78 54	-0.361	-0.371	513

* See footnote 1.

positive sign of V_c and the proportionality of V_c with $\sqrt{c}$ in the most dilute solutions.   It also seems interesting to compare the individuality of heats of dilution within a group of salts of the same valence type—this individuality is predicted by the theory, as each salt will have its own value for 'a'—with the variation of ionic radii as determined from atomic theories and from crystal structure analysis.   A test of the justification for the use of D and dD/dT for the solvent in equation 2 can also be made by employing solvents with different dielectric properties.

Approximate values of the integral heats of dilution to be expected below 0.1 M can be estimated from equation 2.   For 1–1 type salts at 25°C. we may take 490 as a probable value for A. $V_{0.1} = 490\sqrt{0.1} = 155$ calories per mole of salt.   Strictly speaking, integral heats of dilution cannot be measured directly, since the end concentration can never be made exactly zero and the dilution interval is restricted by the dimensions of the calorimetric apparatus and the volume of the solution to be diluted. Considering these factors, it was estimated that the heat effect accompanying a real dilution in the calorimeter described below could be expected to be of the order of only 0.1 to 0.001 calorie for 1–1 type salts below 0.1 M, corresponding approximately to temperature changes of 1×10^{-3} to 1×10^{-6} degree.   These temperature changes should be measured with the greatest accuracy possible, and it was with the above considerations in mind that the calorimeter shown in figure 3 and briefly described below was designed.   For more complete details of construction and operation the reader is referred to other publications (17).

APPARATUS

General considerations

A multiple junction thermoelement, in conjunction with a highly sensitive mirror galvanometer, has proved itself best adapted for the measurement of such small temperature changes. The heat effects produced in the calorimeter by the reaction under consideration must be sharply defined and any secondary effects (heats of absorption, conduction, etc.) must be reduced to a minimum.   Further, disturbing heat effects caused by thermal

contact of the calorimeter with its surroundings should be eliminated as far as possible. The elimination of external disturbances was accomplished (a) by thermal insulation of the calorimeter from its surrounding bath by the use of a Dewar vessel, (b) by employing an adiabatic method, the temperature of the surrounding bath at any moment during the measurement being held as nearly as possible equal to the temperature inside the calorimeter, (c) by using the differential principle. This principle calls for the use of two symmetrically constructed calorimeter vessels placed under similar conditions in a thermally homogeneous medium, whereby any remaining thermal disturbances between outside and inside are duplicated in both vessels. The small heat effects accompanying the dilution are then produced in one of the calorimeter vessels, under otherwise symmetrical conditions, and the temperature differences before and after the reaction are measured as exactly as possible. An unsilvered Dewar vessel was used as the calorimeter vessel and was divided into two symmetrical halves by the multiple junction thermocouple.

The thermocouple

The thermocouples employed for measuring the small temperature differences produced between the two calorimeter halves were built into the removable partitions that divided the Dewar vessels into two symmetrical parts. The individual elements, 1000 to 1500 in number, were distributed over the greater part of the surface of this partition, since the heat produced by stirring in the two calorimeter halves was probably not homogeneously distributed; the number of thermoelements and their distribution, together with other factors to be mentioned, permitted the temperature differences to be measured with an accuracy of 2×10^{-7} degree.

Two forms of large thermocouples are now in use in this laboratory. Some details of their construction are shown in figure 1 and both are fully described elsewhere.

Type 2 is a newer form (18) and has several advantages over the older form (17). The thermoelements are better insulated from the medium in contact with them. The whole is completely

enclosed in a gilded copper case, the sides and top of which are of German silver, giving greater mechanical sturdiness and still allowing the removal of the vaseline and cleaning of the interior when necessary. Type 2 has a slightly greater lag than type 1 in registering temperature differences, but with the methods employed this is no serious objection.

The 1000 or more thermoelements cover a surface of about 110 square centimeters on either side of the partition, an area equal to approximately three-quarters of the surface of the partition and to one-fifth of the surface in contact with liquid in each calorimeter half.

Type 1   Type 2
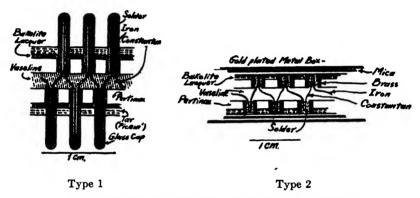
FIG. 1. DETAILS OF MULTIPLE JUNCTION THERMOCOUPLES

The thermal and electrical constants of these thermocouples are discussed below.

The calorimeter vessel

Unsilvered Dewar vessels were used as calorimeters. The following description pertains in particular to a vessel of 2 liters capacity, 200 mm. inner height and 120 mm. inner diameter. Doubts have been frequently expressed as to the advantages obtained by the use of calorimeters with vacuum jackets. Special tests demonstrated clearly the advantages to be obtained.

So that the thermocouples might be removed from the calorimeter when desired they were cemented into Pertinax or hollow German silver frames which had previously been fastened to the

inside of the calorimeter, dividing it into two halves. The frame was cemented to the Dewar vessel with Picein (Portland cement was tried but its use seemed to cause development of strains in the glass and subsequent collapse of the vessel), and the thermocouple, after being pushed into place in the frame, was made tight and fast with a mixture of one part wax and three parts lanolin.
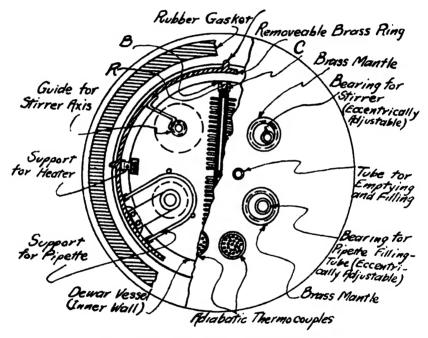
FIG. 2. TOP VIEW OF CALORIMETER VESSEL

The cover is halved

To aid the inserting and removal of the thermocouple an insulated constantan resistance wire was built in between the frame and the thermocouple; electric heating of the wire permitted the easy withdrawal of the thermocouple.

The Dewar vessel was fitted with a lead monoxide-glycerine cement into a flanged brass collar onto which the calorimeter cover, also of brass, was fastened with screw clamps. The vessel
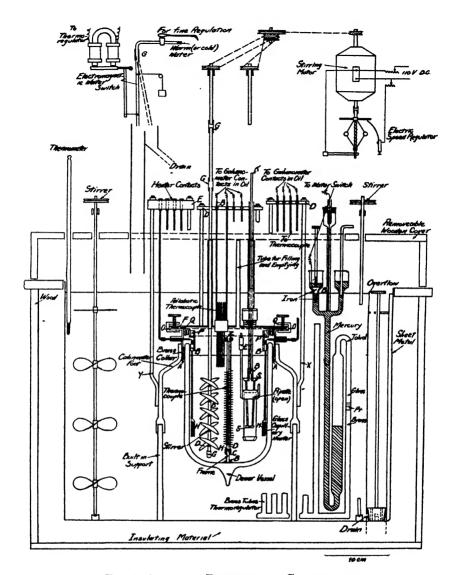
FIG. 3. ADIABATIC DIFFERENTIAL CALORIMETER

A, lead monoxide-glycerine cement; B, Picein; C, lanolin-wax mixture; D, Pertinax; E, hard rubber; F, rubber ring; G, rubber tubing; H, constantan heating wire; O, screw clamps; P, holder for heater; Q, supporting lugs on brass ring; R, Pertinax frame; S, double stopper; X, support for thermocouple leads; Y, support for heater leads.

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and cover are shown in detail in figures 2 and 3 (symmetrical attachments are shown only for one side).

Inside the calorimeter, resting on its brass collar, is a removable brass ring from which were supported (one in each half) heating elements, pipettes and bearings for the stirrer axes.

The heating elements were of constantan enclosed in collapsed glass capillary tubes. Their resistances were matched and remained constant over a period of months to ± 0.01 per cent, independent of the heating current up to 0.5 ampere. To switch
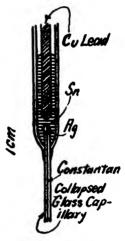
FIG. 4. DETAIL OF CAPILLARY HEATER

the heating current into the heater circuits and to exactly control the time of heating by means of an electric clock a specially devised electric arrangement was used (19, 17). This permitted the time of heating to be determined to within $\frac{1}{100}$ second.

The metal pipettes, recently gilded, served to hold the solutions to be diluted. They could be opened and closed by raising or lowering a rigid, double, ground-metal stopper. The pipettes could also be filled and emptied without being removed from the calorimeter through an opening in the upper stopper, closed or opened as desired by means of a small ground-glass stopper. Thus the volume of the pipettes and the volume of solution to be diluted was always sharply defined.

The stirrers in either half were mirror images of each other and were turned at equal speeds. The constancy of this speed was assured by the use of an electrical speed regulator (19, 17).

Galvanometer and galvanometer readings

A moving-coil mirror galvanometer made by Kipp and Zonen (model Ze) was used to measure the potential developed by the
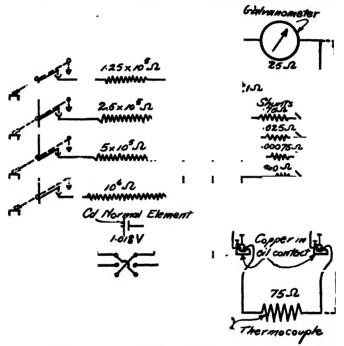
FIG. 5. DIAGRAM OF GALVANOMETER CONNECTION TO THERMOCOUPLE

The diagram shows arrangements for compensating and reducing sensitivity

thermocouple. Its maximum sensitivity was 1.5×10^{-10} ampere per millimeter scale deflection at a distance of one meter. For most of the measurements the sensitivity was reduced to one-eighth of this value by means of an adjustable magnetic shunt. For measurements at the lowest concentrations a sensitivity of about 4.5×10^{-10} ampere per millimeter per meter was used. The internal resistance of the galvanometer was 25 ohms; the

thermocouple connected directly to it had a resistance of about 75 ohms. One binding post of the galvanometer was permanently connected to its case; electrostatic disturbances, otherwise noticeable, were thus eliminated. The leads to the galvanometer were of drilled lead cable, the outer sheathing of which was earthed to further reduce electrostatic and electromagnetic disturbances. The cable was joined to the leads from the thermocouple through contacts of pure copper immersed in water-free mineral oil (17). In general, all contacts were made through pure copper similarly immersed in well-stirred oil thermostats to minimize indefiniteness of contact and stray thermal influences.

To test the sensitivity of the galvanometer during, or before and after, the course of a measurement and to compensate, if necessary, any large galvanometer deflections caused by disturb-
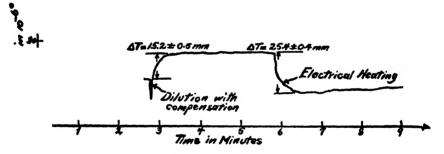
FIG. 6. EXAMPLE OF SEMI-AUTOMATIC ΔT-TIME PLOT DURING A DILUTION WITH ACCOMPANYING WATER VALUE DETERMINATION

ing thermal forces, the arrangement shown in figure 5 was devised. A current of 1×10^{-8} ampere, or stronger currents up to 1.5×10^{-7} ampere, could thus be sent through the galvanometer circuit. With the usual galvanometer sensitivity this corresponded to a deflection of 56.5 mm. at a scale distance of 2.5 meters; this deflection was always reproducible and served to test the working condition of the galvanometer. To protect the highly sensitive galvanometer its sensitivity could be temporarily reduced, up to a millionfold, by means of the four shunts shown.

Galvanometer deflections were observed in the usual way with a telescope; during the course of a measurement readings were

taken at three to four second intervals and plotted on millimeter paper with an accuracy of 0.2 mm. A semi-automatic device was later designed which gave a greater accuracy in the plotting of these readings and relieved the observer of considerable eye strain. A fixed light source (slit with cross thread) fell on the galvanometer mirror and was reflected onto a ground-glass plate 2.5 meters from the galvanometer. This plate was on the sliding carriage of the recording device and was marked with a vertical line. By a suitable gearing device the carriage and a pencil firmly fastened to it could be moved horizontally so that at all times the marker on the plate coincided with the dark thread in the light image. Simultaneously, uniformly rotating rollers carried a piece of millimeter paper under the pencil. A plot so obtained is shown in figure 6.

The outer bath

As is shown later, it was necessary to control the temperature of the water bath surrounding the calorimeter to 0.001°C. or, better, to 0.0001°C. This demanded a thermoregulator with as little lag as possible, a bath well-insulated from the surrounding air, and a rapidly responding heating or cooling source. The thermoregulator (see figure 3) was a comb-shaped system of brass tubes with a surface of 3800 sq. cm. and a volume of 615 cc. to which was attached a glass regulating head. The regulator was filled with toluene which had been freed from sulfur compounds by shaking with mercury and also from air bubbles, and the head contained mercury.

For holding a constant temperature an electric heater was found to have too great a lag. Temperature changes were produced by adding hot or cold water in quantities previously regulated by the fine adjustment of long-handled stop-cocks; electromagnets actuated by the regulator directed this water either into the bath or into a drain, as required. Two and one-half seconds after an addition of hot or cold water, corresponding to a temperature change of 0.001°C., the regulator responded. A temperature constant to within 0.0002°C. could be maintained for hours. To control the temperature difference between the outer bath and the

inside of the calorimeter an 'adiabatic' thermocouple consisting of 24 iron-constantan elements was built into the calorimeter cover; by means of a light image reflected onto a ground-glass scale this temperature difference could at all times be observed and controlled by the addition of hot or cold water.

MANIPULATION OF THE APPARATUS

For one large thermocouple with 1072 iron-constantan thermoelements, assuming an E.M.F. of 5.1×10^{-5} volt per degree per element, the E.M.F. developed per degree temperature difference was 0.0548 volt. With a total resistance of 100 ohms in the galvanometer circuit this corresponded to a sensitivity of 0.6×10^{-6} degree per millimeter deflection at a scale distance of 3.3 meters and the usual setting of the galvanometer's magnetic shunt; a maximum sensitivity of about 1×10^{-7} degree per millimeter deflection could be obtained. Naturally the thermocouple showed a certain lag in responding to temperature differences (for example, after current had been passed through one of the calorimeter heaters for two seconds the galvanometer first began to register this effect four and one-half seconds after the beginning of the heating and showed a maximum deflection, corresponding to the new thermal E.M.F., five or six seconds later), but this effect was of no great importance, since for the graphical determination of temperature differences the ΔT-time plot was taken for some minutes before and after the reaction. The initial and final portions of these plots were linear and practically parallel over a period of several minutes (see figure 6) and could be extrapolated without much error (usually < 0.5 mm.).

From the heat conductivity constant of the Dewar vessel $\left(\dfrac{-d \Delta T}{\Delta T \cdot \text{min.}} \approx 0.004 \right)$ and the constancy of the adiabatic control it was calculated that temperature differences between the outer bath and the interior of the calorimeter of the order of $0.0001°$C. produced a temperature change in the interior of the calorimeter of $\pm 4 \times 10^{-7}$ degree per minute. Since the two halves of the differential calorimeter were practically equivalent with respect to heat conductivity such disturbances were probably reduced ten-

fold as far as they affected the temperature difference between the two halves. For most of the dilutions the temperature change produced was not greater than 1×10^{-4} degree (of the same order of magnitude as the variation in the adiabatic control) and the method of measurement employed was practically 'isothermal-adiabatic' calorimetry. When larger heats of dilution were measured, corresponding to $\Delta T = 1 \times 10^{-3}$ to 1×10^{-4} degree, with compensating heating (see below) in the cooler half of the calorimeter, the adiabatic error was certainly greater but the relative accuracy of the measurements was not decreased. With negative heats of dilution, i.e, absorption of heat, as a consequence of the compensating heating in the same half, very small temperature changes resulted and the adiabatic error was practically zero.

Possible disturbing factors within the calorimeter were the heat developed by stirring and the thermal conductivity between the two halves through the large thermocouple. Stirring was necessary to insure complete mixing of the solution to be diluted with the body of liquid in the calorimeter and the rapid completion of the reaction. The mirror image construction of the two stirrers and their constant speed produced quantities of heat in the two calorimeter halves which were small and equal; the solutions in the metal pipettes were of course always at a somewhat lower temperature than the liquid in the calorimeter as a result of the lag in the transmission of this heat of stirring, but this effect was eliminated by opening both pipettes when a measurement was made (solution to be diluted in one pipette and calorimeter liquid in the other) and diluting their contents simultaneously.

The effect of conductance between the two calorimeter halves was to diminish any temperature difference produced by a reaction in one-half. For small heats of dilution (less than 40 mm. galvanometer deflection) this reduction amounted to about 5 per cent per minute, an amount small in comparison with other sources of error, so that by linear extrapolation of the initial and final parts of the ΔT-time curves the heat effect could be evaluated to within 1 or 2 per cent. For larger heats of dilution it was found desirable to compensate the ensuing temperature differences by electrical heating—for negative heats of dilution by

heating in the reaction half of the calorimeter and for positive heats by heating in the other half.

To reduce heat effects produced by absorption on the inner wall of the Dewar vessel in some runs the inside of the calorimeter was covered with a thin layer of pure vaseline. However it is believed that such effects in general were not present (20).

The evaluation of such ΔT-time curves as shown in figure 6 assumes that the heat effect produced by the simultaneous opening of both pipettes is produced by the dilution reaction alone. To test this, frequent controls were run; both pipettes, filled with water or with the same solution, were opened together and curves
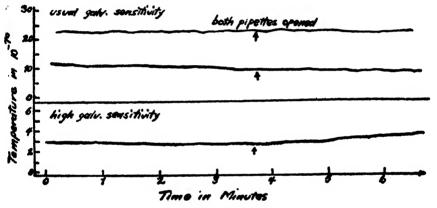
FIG. 7. TYPICAL BLANK RUNS

such as those shown in figure 7 were obtained. The complete absence of effects due to the pipette temperature lagging behind the main calorimeter temperature was thus shown.

Electrical calibration was employed to evaluate the galvanometer deflections produced by dilution reactions in terms of calories. By means of the switching device mentioned above, current was passed through one of the calorimeter heaters for a definite time (two seconds or some multiple thereof) and the galvanometer deflection was obtained in the usual way. A knowledge of the time of heating, resistance of the heater and applied potential permitted an exact evaluation of the galvanometer readings in terms of calories.

RESULTS OF MEASUREMENTS

The experimental results are shown in figures 8, 9, 10 and 11. The extrapolation of the curves from the lowest measured con-
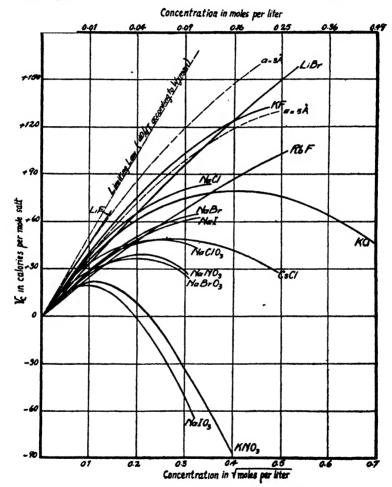
FIG. 8. INTEGRAL HEATS OF DILUTION OF 1–1 SALTS AT 25°C.

LiF (21), LiBr (8), NaCl (22), NaBr (22), NaI (23), NaClO₃ (24), NaBrO₃ (24), NaIO₃ (24), NaNO₃ (24), KF (8), KCl (25), KNO₃ (26), RbF (26), CsCl (8).

centration $(1 \times 10^{-4}$ to $1 \times 10^{-5} M)$ to zero concentration is made without any great error; an uncertainty of perhaps one calorie is

introduced into the V_c values by this extrapolation. For the
various types of salts the limiting law is also shown (the dielectric
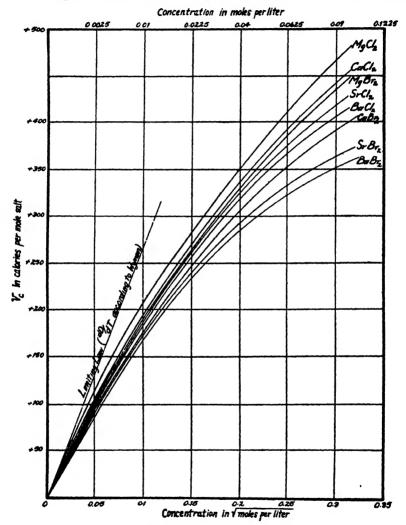
FIG. 9. INTEGRAL HEATS OF DILUTION OF 2-1 SALTS AT 25°C.

MgCl$_2$, CaCl$_2$, SrCl$_2$, BaCl$_2$, MgBr$_2$, CaBr$_2$, SrBr$_2$, BaBr$_2$ (27)

constant measurements of Wyman were used in the calculations);
for the 1-1 type theoretical curves for V_c calculated from equation
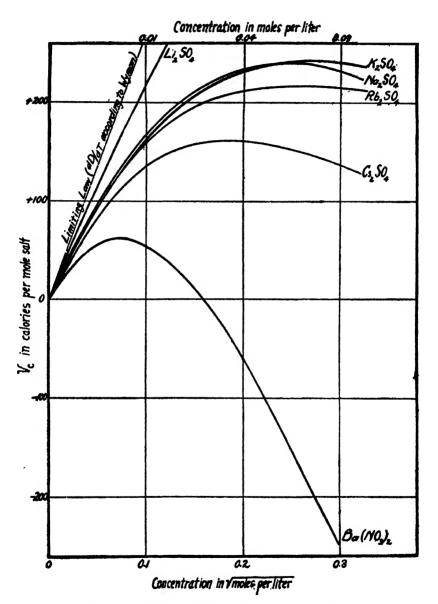
FIG. 10. INTEGRAL HEATS OF DILUTION AT 25°C.
Li₂SO₄, Na₂SO₄, K₂SO₄, Rb₂SO₄, Cs₂SO₄, Ba(NO₃)₂ (28)

2 for 'a' values of 3 and 5 Ångström units are also given. For 2–1 (27) and 1–2 type salts the theoretical value of A is 5.2 times as large as for 1–1 salts; for 2–2 type salts the ratio is 8 to 1. A discussion of the causes of the deviations of the observed values from the calculated is given later, although it may be observed at once that the cutting of the curves for salts of the same valence type with a common and variable ion and the appearance of negative values for V_c at moderate dilutions are in complete dis-
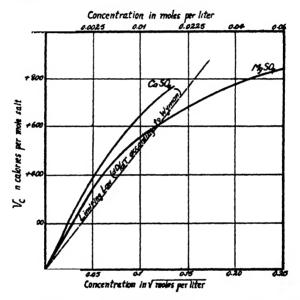
FIG. 11. INTEGRAL HEATS OF DILUTION OF 2–2 SALTS AT 25°C.
CaSO$_4$ (26), MgSO$_4$ (8)

agreement with the simple interionic theory with its various assumptions noted above.

From the measured integral heats of dilution it is possible to calculate directly the differential heats of dilution for each salt for the measured concentration range. These differential heats of dilution are necessary for calculating the temperature coefficient of activity and osmotic coefficients (27, 29) and are particularly useful in the calculation of activity coefficients at room temperature from freezing point data. A knowledge of the integral heats of

dilution is necessary in order to make proper corrections of measured heats of precipitation (30) and neutralization (31). Where a heat of solution is to be extrapolated to infinite dilution from some finite concentration the integral heat of dilution is required; this is illustrated in the calculation of ionic entropies (32). This heat of solution at infinite dilution can also be used to test Born's calculation of the lattice energy of solids (33). Although the measurements discussed in this paper were obtained for concentrations in general below 0.1 M, such measurements are easily combined with measurements in more concentrated solutions to obtain curves for the complete solubility range (20).

THEORETICAL DISCUSSION

For all electrolytes on which measurements have been made, positive values for V_c have been found at the lowest concentrations. There can be no doubt that this is true for all electrolytes at sufficiently great dilutions. The theory, with its various assumptions, demands positive V_c values throughout the entire concentration range for completely dissociated electrolytes.

At the lowest measured concentrations the integral heats of dilution increase more or less proportionally with the square root of the concentration expressed in moles of salt per liter of solution (there is no appreciable difference between weight and volume concentrations in this range). This proportionality is found to hold on the average for 1-1 and 2-1 type salts up to concentrations of about 0.01 M, although for individual salts this limit may be higher or lower.[3] The slopes found agree well with the theory, considering the uncertainty in dD/dT.

For the various salts measured (this is presumably true for all electrolytes) the V_c values remain individual down to very low concentrations; for 2-1 salts this individuality is still definitely recognizable below $1 \times 10^{-4} M$. This does not agree with the limiting law of the Debye-Hückel theory, according to which all strong electrolytes of the same valence type should have identical

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[3] The proportionality with $\sqrt{c}$ is better for the 1-1 type salts. The alkaline earth chlorides and bromides show a closer approach to this proportionality than the alkali sulfates and alkaline earth nitrates.

heats of dilution, but it is not in contradiction with the more exact development of the theory (9, 10) in view of the individuality of the 'a' parameter, if for no other reason.

If the different 'a' values of the various salts (they are assumed to be independent of temperature[4]) are alone responsible for the individuality observed, then in any one valence type the electrolytes with the smallest 'a' values should have the highest V_c values. Interpreting the measurements in these terms it is seen that $a_{LiF} < a_{KF} < a_{RbF}$; $a_{NaCl} < a_{NaBr} < a_{NaI}$; $a_{MgCl_2} < a_{CaCl_2} < a_{SrCl_2} < a_{BaCl_2}$; $a_{MgBr_2} < a_{CaBr_2} < a_{SrBr_2} < a_{BaBr_2}$; $a_{Mg(NO_3)_2} < a_{Ca(NO_3)_2} < a_{Sr(NO_3)_2} < a_{Ba(NO_3)_2}$; $a_{Li_2SO_4} < a_{Na_2SO_4} < a_{K_2SO_4} < a_{Rb_2SO_4} < a_{Cs_2SO_4}$. This would seem to indicate a parallelism between the apparent diameter of ions in solution and the ionic radii as determined from crystal structure analysis. On the other hand the measurements would have to be interpreted as showing $a_{MgSO_4} > a_{CaSO_4}$.[5] In other words, there is not always a parallelism between 'a' and the ionic radius, assuming that the observed slopes A can be interpreted as being caused by different 'a' values alone. It might also be mentioned that even if the observed A values had indicated 'a' values in the inverse order as compared with ionic radii in crystals, as for instance in the case of the alkaline earth sulfates just mentioned, the explanation could be advanced that the smaller ions actually have a larger apparent diameter in solution due to their greater hydration (16). But the comparison of magnesium and calcium ions gives opposing pictures depending on whether these ions are associated with halogen

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[4] By measuring the temperature coefficient of the potential of the cell

| Zn-Hg | | ZnSO$_4$ | | PbSO$_4$(solid) | | Pb-Hg |
| Two phase amalgam | | M | | | | Two phase amalgam |

LaMer and Cowperthwaite have obtained evidence that 'a' for zinc sulfate is independent of temperature from 0° to 50°C. Professor LaMer has kindly given us this information. Similar results were obtained by him for cadmium sulfate. It seems doubtful that this can be true for all electrolytes. Measurements of the heat of dilution of potassium nitrate and of potassium chloride at 25°C. and 12.5°C. indicate that 'a' for these salts varies with the temperature (16).

[5] Recent measurements below $4 \times 10^{-3} M$ indicate that at very small concentrations $a_{CaSO_4} > a_{MgSO_4}$.

ions or a sulfate ion.   The nature of the common ion, as well as the relative values of the ionic radii, seems to determine the relative values of 'a'.

A similar interpretation of activity coefficients determined by E.M.F. or freezing point methods should give the same order for the 'a' values in a series of salts with a common ion, since the theory would obviously require the use of the same 'a' in the theoretical activity coefficient and V_c expressions.   Actually, however, this is not the case.   Considering the alkali halides, for instance (34), it is found that the salts with higher V_c, and therefore smaller 'a', values have the higher activity coefficients, i.e., apparently the larger 'a'.   The same anomaly is shown by the alkaline earth chlorides.   According to V_c measurements $a_{CaCl_2} < a_{SrCl_2} < a_{BaCl_2}$, while the activity coefficients (35) give $a_{CaCl_2} > a_{SrCl_2} > a_{BaCl_2}$.   It is not impossible that if the activity coefficient measurements could be extended to the same dilution with the same accuracy as the V_c measurements, the observed discrepancies would disappear.   But considering the unexpected order of 'a' values already found among the alkaline earth sulfates, it seems more probable that the properties of these electrolytes cannot be explained in terms of 'a' values alone, even in solutions whose concentration is only 0.01 M.[6]

Above 0.01 M the situation is even more complicated.   The V_c curves bend toward the abscissa and in some cases (e.g., $NaIO_3$, KNO_3, $Ba(NO_3)_2$) even assume negative values at concentrations below 0.1 M.   This bending towards negative values is to be expected from the theory, but in most cases is greater than anticipated (LiBr, RbF, and perhaps LiF are exceptions).   The curves of salts with a common and a variable ion cut each other in several instances; this too cannot be explained by any present treatment of the Debye-Hückel theory.

Evidently other factors than those considered by equation 2 are responsible for the anomalies mentioned.   The existence of undissociated molecules in solutions of strong electrolytes even at concentrations as low as 0.01 M is a possible explanation for the

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[6] A more complete comparison of 'a' values indicated by heat of dilution and activity coefficient measurements is given in Naturwissenschaften **19**, 359 (1931).

pronounced trend toward negative values (36) of V_c. Above 0.01 M it is possible to explain qualitatively the bending toward negative values as a dissociation effect superposed on the interionic effect. An attempt to find quantitative support for this suggestion gave no definite results. Since the effect of any undissociated portion of a salt should be approximately proportional to the concentration (24), and since salts are probably largely dissociated in 1 M solutions, assuming a molar heat of dissociation of the order of magnitude of 10 kilogram-calories, the contribution of the heat of dissociation to the integral heat of dilution in a 0.01 M solution would probably be less than 10 calories, an amount scarcely sufficient to account for the observed bending of the V_c curves at this concentration. On the other hand the assumption of the presence of undissociated molecules cannot be disproved; the evidence as to their existence is conflicting (37).

Formally it is possible to explain the bending of the V_c curves by the addition of other terms to equation 2, for example, a term involving da/dT. The theoretical treatment of V_c is thereby considerably complicated and no exact expression has as yet been derived in terms of the Gronwall, LaMer and Sandved calculations. However, the original Debye-Hückel treatment involving the parameter 'a' goes over into this form for large values of 'a', and it seems useful to investigate the effect of a term involving da/dT for the simpler form of the theory. Gross and Halpern (2) have shown that the added term is proportional to the concentration and negative. Bjerrum (5) has shown that the results of Richards and Rowe at higher concentrations can be explained on this basis if da/dT has small positive or negative values, but does not place any great reliance on this explanation, as at such concentrations the simple interionic theory cannot be expected to be valid. Our results at lower concentrations can also be formally explained by adding a term in da/dT, but, as in the case of the assumption of the existence of undissociated molecules, since the effect is proportional to the concentration it must be negligible at concentrations where the individuality of the V_c curves still persists, to judge from individual deviations at higher concentrations.

Another possibility to be considered is the dependence of the

dielectric constant on the concentration of the salt. The dielectric constants of aqueous salt solutions certainly differ from the dielectric constant of pure water, and presumably dD/dT has an individual value for each salt. Approximate calculations (38) however, have shown that this influence would be proportional to some power of the concentration higher than the square root so that, as in the case of the factors already discussed, the bending of the V_c curves might be explained, but not their individuality at
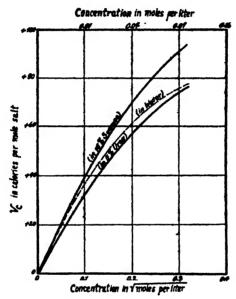
FIG. 12. INTEGRAL HEATS OF DILUTION AT 25°C.
KCl in water (25), KCl in 5% sucrose (39), KCl in 15% urea (39)

such dilutions. Measurements of the dielectric constants of solutions of electrolytes are difficult, and the experimental results obtained thus far are too discordant to be applied to any calculation. The heats of dilution of potassium chloride in 15 per cent urea and 5 per cent sucrose solutions have been measured (39) to see whether the known D and dD/dT values of these solutions (40) would measurably affect the heat of dilution. As is seen from figure 12 the deviations are comparatively small, although from

the constants for the urea solutions ($D = 82.8$, $dD/dT = -0.20$) an initial slope of -228 was to be expected theoretically, i.e., a negative heat of dilution. Either the dielectric constants are subject to large errors, or, as a first approximation, the D and dD/dT values for pure water are to be used in these cases and the observed deviations are to be explained as caused by one or more of the factors discussed above.

It follows from the V_c measurements that the limiting law expression of Debye and Hückel for great dilution is verified not only with respect to the sign of V_c and its approximate proportionality with $\sqrt{c}$, but also with respect to the numerical values of the initial slopes A, considering the uncertainty in the theoretical values of the dielectric constants. It seems though that the concentration range in which the relatively simple relations of the limiting law are valid lies far below 0.01 M. Above this limit other effects are certainly present, as indicated by the abnormal bending of the V_c curves toward negative values and the cutting of individual curves. Three possible effects which have been discussed have an action which increases in proportion to the concentration, and if several are present simultaneously it is impossible to predict anything definite about the relationship between V_c and the concentration. It is proposed to extend the measurements to still lower concentrations and to other types of salts, particularly salts of high valence types, to see how far the individual behavior of salts of a definite type persists. The theory of Debye and Hückel at the present time seems to be a good approximation theory, but the individual behavior of electrolytes at such small concentrations means that an exact knowledge of activity coefficients, osmotic coefficients, heats of dilution, etc., can be obtained only by direct measurement.

<div align="center">SUMMARY</div>

A differential adiabatic calorimeter is described which permits the measurement of small temperature differences with an accuracy of 2×10^{-7} degree.

The integral heats of dilution of fourteen 1–1 type salts,

twelve[7] 2–1 type salts, five 1–2 type salts, and two 2–2 type salts have been measured down to concentrations of about $1 \times 10^{-5} M$ with an average error of 2 per cent.

Below 0.01 M in general the integral heats of dilution are positive in sign (heat is evolved by the dilution), are more or less proportional to the square root of the concentration, and agree fairly well with the slopes calculated from the limiting law of the Debye-Hückel theory, considering the uncertainty existing in the dielectric constant data. The individuality of the integral heat of dilution persists down to the lowest measured concentrations.

Above 0.01 M in general the abnormal bending toward negative values and the cutting of individual curves indicates the influence of factors other than a simple interionic effect. Three possible factors are discussed.

A comparison of the order of the heats of dilution in the alkali and alkaline earth halides with the order of the activity coefficients of these salts shows anomalies even at low concentrations.

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AZOXY COMPOUNDS

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I. THE ISOMERISM AND PROPERTIES OF AZOXYBENZENE
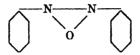
Azoxybenzene was first made by Zinin (1) in 1841 by reducing nitrobenzene with alcoholic potassium hydroxide. It was shown by him to be an intermediate product in the reduction of nitrobenzene to aniline, and was given the formula $C_6H_5N_2OC_6H_5$.

Since its discovery a very large number of derivatives have been prepared and their chemical properties have been studied. Considering the nature of other products of the reduction of nitrobenzene—nitrosobenzene, phenylhydroxylamine, azobenzene and hydrazobenzene—the stable nature of azoxy compounds is remarkable. They are not attacked by dilute nitric, sulfuric, or hydrochloric acid, or by sodium hydroxide solution. They are, in general, resistant to the action of oxidizing agents. They show considerable stability toward many reducing agents. They are not attacked by hydroxylamine or by phenylhydrazine, and many other reagents have no action upon them. They appear, in fact, to be an intermediate stage in the reduction of nitrobenzene derivatives to amines, with an unexpected degree of stability.

Naturally the possibility of isomerism in the case of azoxy compounds has interested many chemists; since a discussion of these compounds is vitally dependent on their structure this topic will first be considered.

The early representation of the molecule of azoxybenzene suggested by Kekulé, indicated two phenyl groups connected

through the agency of a three-membered ring composed of two nitrogen atoms and one oxygen atom as follows:

This formula, as is obvious, contains trivalent nitrogen atoms. It had no experimental basis but was arbitrarily selected.

In 1870, Melms (2) succeeded in making p,p'-azoxytoluene, melting at 70°C., by reducing p-nitrotoluene with sodium amalgam in an alcoholic solution. Janovsky and Reimann (3) later reported a second p,p'-azoxytoluene, melting at 75°. The authors considered it to be isomeric with the compound made by Melms, which they called the α form; they called their own compound the β form. The inclusion in Beilstein (3a) of the work of Bamberger and Renauld (4) under the β compound suggests that the Janovsky and Reimann compound was also made by these authors. Reference to the original papers, however, shows the one made by them to have a melting point of 69°. No doubt this compound is identical with that of Melms. Cumming and Ferrier (5) were unable to obtain the isomer melting at 75° by either of the methods used by Janovsky and Reimann, and have further shown that it is not a solid solution of p,p'-azotoluene in p,p'-azoxytoluene. They believed the existence of this β isomeride "extremely doubtful." Further Hantzsch and Werner (6) stated that H. Goldschmidt in a private communication reported that he had not been able to substantiate the claim of Janovsky and Reimann.

The work of Melms had seemed to suggest the possibility of isomerism among azoxy compounds. The same year Kekulé and Hidegh (7) obtained, by the action of phosphorus pentachloride on p-monohydroxyazobenzene, a compound which they believed to be p-monohydroxyazoxybenzene. These investigators gave no melting point, but the work of Wallach and Kiepenheimer (8) established it as 145°. The fact that the compound formed p-monohydroxyazobenzene on treatment with alcohol and sodium seemed to support the view of Kekulé and Hidegh regarding its

constitution, but its insolubility in alkalies and its failure to react with acetic anhydride even at 180° to 200° denied this probability. Later Bamberger (9) prepared a p-monohydroxyazoxybenzene by condensation of p-nitrosophenol and phenylhydroxylamine and found it to melt at 156.5°. Later still Angeli prepared both the α and β isomers of this compound: the α isomer had a melting point of 156° and was identical with the compound prepared by Bamberger; the β isomer had a melting point of 117°. The compound which Kekulé and Jidegh prepared is not identical with either of these and cannot be considered as an isomeric p-monohydroxyazoxybenzene. So far as we have been able to find, no further investigation of this compound has been made.

Up to 1890, then, only one case of apparent isomerism was known, namely, that of p,p'-azoxytoluene. Early in that year Hantzsch and Werner (10), in an extended consideration of the isomerism of nitrogen compounds, suggested that azoxy compounds might illustrate stereoisomerism of the geometric type, quite closely analogous to the suggested isomerism of the azo compounds. Between the nitrogen atoms there might be considered to be two bonds, one of them through an intermediate oxygen atom. They represented the two isomeric p,p'-azoxytoluenes by the formulas

$$O \underset{N-C_6H_4CH_3}{\overset{N-C_6H_4CH_3}{\Big\langle}} \quad \text{and} \quad \underset{N-C_6H_4CH_3}{\overset{CH_3C_6H_4-N}{\Big\rangle}} O$$

The authors suggested the formulas with little comment. They did not suggest, as was later pertinently pointed out by Angeli, that such isomers, if of the usual type, should be capable of transformation one into the other through the agency of heat, bromine, acid, etc.

In 1900 Bamberger, through the action of aqueous sodium hydroxide on nitrosobenzene (11) and later (12) by the action of light on a benzene solution of nitrosobenzene, found what he at first considered as two o-monohydroxyazoxybenzenes. One melted at 75.5°–76°; the other, which he called iso-o-hydroxyazoxybenzene, melted at 108°–108.5°. He came to the conclu-

sion (9) that only one was an azoxy compound, the other being orthoquinoid in character. Later, however, he suggested that they might possibly be *cis* and *trans* isomers (13). This is particularly interesting, for later Bigiavi and Poggi (14) established the fact that both of these were azoxy compounds and isomeric with one another.

In 1901 Wacker (15) made α-azoxynaphthalene by the reduction of α-nitronaphthalene with ammonium chloride and zinc dust, and from α-nitronaphthylhydroxylamine. He found two products: one was yellow and melted at 127°; the other was red and melted at 126.5°. Both belonged to the rhombic system. He noticed further that light turned the yellow compound into the red one. He considered both to be α-azoxynaphthalenes. Here then we have what may possibly be considered a case of geometrical isomerism. Cumming and Steel (16) in 1923 reported the formation of two azoxynaphthalenes from α-nitronaphthalene dissolved in alcohol to which ammonium chloride was added, followed by the gradual addition of zinc dust. They found both the yellow and red modifications of Wacker; both melted at 127°. The yellow form became red in the sunlight. They believed the change from the yellow to the red modification to be represented by a change of structure
from

$$O \Big\langle \begin{array}{c} N-R \\ | \\ N-R \end{array}$$

to

$$R-N \!=\! N-R \\ \| \\ O$$

The absorption coefficients showed that the two forms were not dimorphous. It should be remarked, however, that Baudisch and Fürst (17) have studied the action of light on α-azoxynaphthalene and have shown that α-naphthaleneazo-α-naphthol, melting at 224°, is formed. Furthermore, in a later communication, Cumming and Ferrier (18) in a study of the action of light on

azoxy compounds reported a private communication from Cumming and Steel stating that the latter had found that, on long exposure to light, α-azoxynaphthalene gives the α-naphthalene-azo-α-naphthol, melting at 224°, found by Baudisch and Fürst. It is possible, then, that they mistook a transformation into a hydroxyazo compound for a stereoisomeric change.

In 1909 Reissert (19) undertook a further study of azoxy compounds with the possibility of stereoisomerism in view. He assumed that compounds made at an elevated temperature would give only the isomer stable at that temperature, while at a lower temperature he might get pairs of such isomers. With this in view he used a water alcohol solution of sodium hydroxide on nitrosobenzene, the mixture being ice-cooled. He obtained ordinary azoxybenzene melting at 36° and, in small quantities, an isomeric form melting at 84°. By condensation of phenylhydroxylamine with nitrosobenzene under similar conditions he got no trace of the new isomer. With o-nitrosotoluene, reduced as above, he got the usual o-azoxytoluene, melting at 59–60°, and an isomeric isoazoxytoluene melting at 82°. With the corresponding *para* compound he got only the usual *p*-azoxytoluene melting at 70°.

These iso isomers, in both cases, according to Reissert, go gradually into the better known isomers with increase of temperature. He found no fixed transition point; however, the higher the temperature the higher the rate of transition.

Angeli (20) reported that he had been unable to corroborate the work of Reissert in the preparation of isomers of azoxybenzene at low temperatures. His method, however, was not a duplication of that of Reissert's, but consisted in the oxidation of azobenzene with peracetic acid. Nevertheless one might reasonably expect that, if the condition governing the formation of the two isomers is that of definite temperature, the isomers might be expected, whatever the method of preparation.

The work of Reissert, however, is another case in which there is evidence of stereoisomerism of the geometrical type. Still another case has recently been observed by Cumming and Ferrier (21). These experimenters prepared β,β'-azoxynaphthalene by

reducing β-nitrosonaphthalene with zinc dust and ammonium chloride in alcohol. Their product melted at 164°. Meisenheimer and Witte (22) gave a melting point of 167–168°. Cumming and Ferrier exposed their compound to light and obtained a red compound melting at 162°; this change is irreversible. In view of the fact that Knipscheer (23) has shown that azoxybenzene is converted by light into o-hydroxyazobenzene and Baudisch and Fürst (17) have shown that α-azoxynaphthalene is changed into α-naphthaleneazo-α-naphthol, the authors tried to show the presence of a hydroxyl group in their compound by benzoylation and methylation, but failed. Support for the assumption of its presence is given, however, by a comparison of the absorption spectra of both their yellow and red isomers with that of β-naphthaleneazo-β-naphthol. Both the red isomer and the β-naphthaleneazo-β-naphthol produced a band with its head at a frequency approximately 1950 units, which was not shown by the yellow isomer; and, furthermore, the characteristic absorption bands of the yellow isomer were not shown by the red. It would, then, seem doubtful that two isomeric azoxy compounds are here described. The color of the red isomer is noteworthy. Azoxy compounds are not deeply colored; they vary, in general, from pale yellow to golden. What structural change due to stereoisomerism could cause such a change in color is not clear. The formation of an azo compound by the action of light gives a perfectly satisfactory explanation of the color change.

We have grouped together the various examples of isomers, which are found in the literature, whose isomerism might be other than structural. There is an element of doubt about the existence of most of them. Probably all of them should be studied further, but most particularly those obtained by Reissert. This author's work should be repeated with care.

There still remains the possibility of structural isomerism, and it is this field which has proved so productive in recent years in the study of the structure of azoxy compounds. Its development is due to Angelo Angeli of the University of Florence, Italy, and to his students. According to his view no isomerism is to be expected in the case of symmetrical compounds of the type of

azoxybenzene (fig. 1) but with a compound like benzeneazoxy-*p*-nitrobenzene two isomers might be expected if the oxygen atom is attached to one nitrogen only, it being pentavalent. They would differ according as the single substituting group were in the ring adjacent to the trivalent or to the pentavalent nitrogen. Whether geometrical isomerism may occur or not there can be no doubt of the existence of structural isomers among the azoxy compounds when they are unsymmetrical. That such isomers were not early observed was due to the methods by which azoxy compounds were made. Such methods consisted in the reduction

Azoxybenzene

α Isomer   β Isomer
m.p. 152°   m.p. 148°

Benzeneazoxy-*p*-nitrobenzene (or *p*-nitroazoxybenzene[1])

FIG. 1. AZOXYBENZENE AND ISOMERS OF BENZENEAZOXY-*p*-NITROBENZENE

of compounds containing a nitro group and, of necessity, led to symmetrical compounds.

The genesis of Angeli's representation of the isomerism of azoxy compounds is probably found in the discussion of the structure of the so-called nitrosophenylhydroxylamine discovered by A. Wohl (24) and a little later by E. Bamberger (25). Both of these investigators prepared it by the action of nitrous acid on phenylhydroxylamine and both gave to it the formula,

[1] Two systems of nomenclature of azoxy compounds are found in the literature. In figure 1 the two isomeric nitro substituted azoxybenzenes are called α and β *p*-nitroazoxybenzene. They are also called α and β benzeneazoxy-*p*-nitrobenzene. We prefer the latter system, though the former is more common. We have used both systems in this paper, largely dependent on their use by the author whose work is being discussed.

$$C_6H_5N\left\langle\begin{array}{l}NO\\OH\end{array}\right.$$

In 1896 Angeli (26) published a new method for the preparation of this interesting substance by condensing nitrobenzene with hydroxylamine and, from its method of preparation, gave it the formula

$$C_6H_5N\!=\!N\!-\!OH$$

This compound, melting at 58.5°, is identical in all respects with that prepared by Wohl and Bamberger and is isomeric with diazobenzolic acid, melting at 46°.   There has been a great deal of discussion concerning the structure of these two compounds which it is not necessary to detail.   It is of interest here as the probable source of the idea that azoxy compounds of dissimilar aryl groups might be expected to exhibit isomerism if connected by the grouping.

$$\begin{array}{c}-N\!=\!N-\\ \parallel\\ O\end{array}$$

Later in the same year Bamberger and Ekecrantz, in a discussion of the compound formed by the methylation of nitrosophenylhydroxylamine, suggested that its formula might be

$$C_6H_5\!-\!N\!\!-\!\!\!-\!\!\!-\!N\!-\!OCH_3$$
$$\diagdown\diagup$$
$$O$$

and in a footnote stated that it is purely arbitrary whether the N_2O group be represented as in the above formula or by the symbol

$$\begin{array}{c}-N\!=\!N-\\ \parallel\\ O\end{array}$$

and that the same is true for azoxybenzene.   On the strength of this note some would give to Bamberger the credit for the suggestion of this structure for azoxybenzene (19).   This would hardly seem justified in view of Angeli's work some months earlier.   Indeed, the application of this formula to azoxy compounds

had hardly been thought worthy of consideration by Bamberger, since four years later, though he was the first to discover two thoroughly authentic isomeric azoxy compounds—the two benzene-azoxy-*o*-phenols—he did not see in them confirmation of this theory of the structure of azoxy compounds but gave to one the quinoid formula.

During the following ten years many azoxy compounds were made, all of them symmetrical in type. In 1906 Angeli and Marchetti (27) studied the reaction of sodium on a mixture of nitrobenzene and aniline. They obtained a compound containing sodium which was readily hydrolyzed yielding an appreciable quantity of azoxybenzene. Similarly, the condensation of α-naphthylamine and α-nitronaphthalene in the presence of sodium gave α-azoxynaphthalene. When, however, they condensed in this way aniline and α-nitronaphthalene, or nitrobenzene and α-naphthylamine, they obtained two isomeric compounds probably represented by the formulas

$$C_6H_5N{=}NC_{10}H_7 \qquad and \qquad C_6H_5N{=}NC_{10}H_7$$
$$\overset{\|}{O} \qquad\qquad\qquad \overset{\|}{O}$$

The condensation of amines with nitro derivatives in the presence of sodium they considered as general in character. Among others they gave methods for the preparation of the isomeric compounds nitrosophenylhydroxylamine and diazobenzolic acid as follows:

$$C_6H_5NO_2 + H_2NOH \rightarrow C_6H_5N{=}NOH$$
$$\overset{\|}{O}$$

$$C_6H_5NH_2 + O_2NOC_2H_5 \rightarrow C_6H_5N{=}NOH$$
$$\overset{\|}{O}$$

It would seem then that the possibility of structural isomerism among azoxy compounds was established in the mind of Angeli at that time. The difficulty of preparing such compounds, however, was great. The condensation of aniline with nitrobenzene does not take place readily. Various attempts to condense the

two substance had been made with condensing agents other than
sodium but without success.   Another method had to be found if
a real advance were to be made.   The promising method of
Bamberger (28)—the condensation of nitrosobenzene and a sub-
stituted phenylhydroxylamine—did not give results.   Instead,
two symmetrical compounds were formed as follows:

$$C_6H_5NO + p\text{-}BrC_6H_4NHOH \rightarrow BrC_6H_4N_2OC_6H_4Br + C_6H_5N_2OC_6H_5$$

As a consequence Angeli sought to oxidize unsymmetrical azo
compounds.   H. Petriew (29) had obtained trinitroazoxytoluene
by treating azotoluene with strong nitric acid, the azo group being
oxidized but the ring attacked as well.   Azobenzene, too, treated
with an acetic acid solution of chromic acid in an enclosed tube at
150° to 250° gave azoxybenzene.   It was, however, found later
by Angeli that this reagent had occasionally the power of trans-
forming an azoxy compound into its isomer.   These facts made
both of these reagents undesirable as oxidizing agents for the azo
group alone.   Angeli (30) therefore studied the action of perace-
tic acid—made by combining glacial acetic acid with 30 per cent
hydrogen peroxide—on azobenzene and found that, on standing
for several days, azoxybenzene was obtained.   It is this reaction
between peracetic acid and azo compounds which has made pos-
sible the extended study of azoxy compounds since 1910.

The first work was done by Angeli and Alessandri (31) on p-
nitroazobenzene.   Treating this with peracetic acid they ob-
tained two isomeric forms: one, that obtained by Zinin (32),
melted at 152°; the other had a melting point of 148°, which later
(33) was given as 149°.   They suggested that these are structural
isomerides; since one of them—that with melting point 152°—is
the older and formed by the action of nitric acid on azoxybenzene
it should be called the α form; the other was called the β form.
These isomers with their melting points are shown in figure 2;
the differences in their constitution are not shown.

Shortly after, Angeli and Valori (34), by oxidizing p-bromoazo-
benzene with hydrogen peroxide and glacial acetic acid, obtained
two isomeric compounds, one melting at 73°, the other at 92°.
They are shown in figure 2 without constitutional differences.

The former is identical with that made by treating azoxybenzene with bromine and is called the α form. Neither of the pairs shown in figure 2 can be transformed, the one into the other, by the usual methods effective with stereoisomers of the geometrical type. They are, however, both reduced to the same azo compound.

To determine the particular structure of each of these pairs it was necessary to consider their chemical action toward various reagents. Both of the α forms were unaffected by nitric acid of

α Forms β Forms

m.p. 152°   m.p. 148°

p-Nitroazoxybenzene

m.p. 73°   m.p. 92°

p-Bromoazoxybenzene
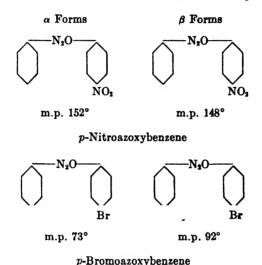
FIG. 2. ISOMERIC FORMS OF p-NITROAZOXYBENZENE AND p-BROMOAZOXYBENZENE

specific gravity 1.45, but both the β forms were nitrated forming respectively p,p'-dinitroazoxybenzene and p-bromo-p'-nitro-azoxybenzene. So, also, treated with bromine, the α forms were unattacked, but the β forms gave p-bromo-p'-nitroazoxybenzene and p,p'-dibromoazoxybenzene, respectively. This action is expressed in figure 3.

In the formula of azoxy compounds suggested by Angeli one of the nitrogens is trivalent, the other pentavalent. To determine the position of each of these, Angeli compared the action of nitric acid and of bromine on azo compounds. p-Bromoazobenzene

acted on by bromine gave p,p'-dibromoazobenzene and with nitric acid it gave p-bromo-p'-nitroazobenzene; in both cases the unsubstituted ring was attacked. So also p-nitroazobenzene with bromine gave p,p'-bromonitroazobenzene and with nitric acid it gave p,p'-dinitroazobenzene. In the case of both com-
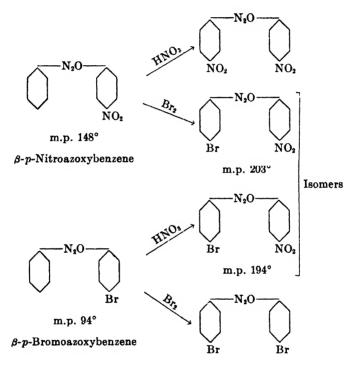
Fig. 3. Action of Nitric Acid and of Bromine on the β Forms of p-Nitro-azoxybenzene and of p-Bromoazoxybenzene

pounds treated with bromine or nitric acid substitution occurred in both rings as shown in figure 4.

In all these cases the bromo or nitro group entered a ring *para* to a trivalent nitrogen. Many other examples could be given to illustrate this fact. It would seem, therefore, a fair assumption that when a single bromo or nitro group enters the ring of an azoxy compound it does so in a ring which is bound to a trivalent nitrogen atom.

On this assumption the well known benzeneazoxy-*p*-nitroben-
zene, more commonly called *p*-nitroazoxybenzene, and which
Angeli calls the α isomer, must have its nitro group in the ring
attached to a trivalent nitrogen, and the oxygen attached to the
pentavalent nitrogen must be adjacent to the second ring; so
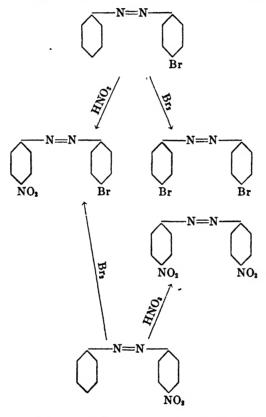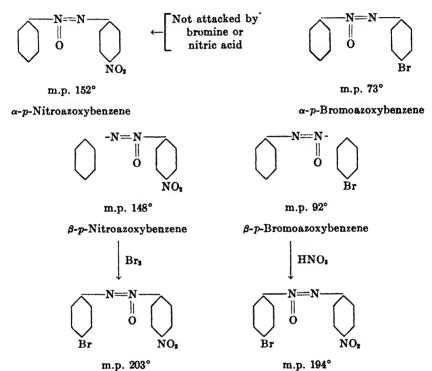
FIG. 4. ACTION OF BROMINE AND OF NITRIC ACID ON *p*-BROMOAZOBENZENE AND ON
p-NITROAZOBENZENE

with others which contain a single substituting group. On
that assumption we can represent the structural formulas of the
various isomers in figures 2 and 3 which contain bromo and nitro-
groups as shown in figure 5.

The two *p*-bromo-*p'*-nitroazoxybenzenes in figure 5 were pre-

pared in 1912 by Angeli and Valori (34). α-p-Bromo-p'-nitro-azoxybenzene, m.p. 194°, was obtained by oxidizing p-bromo-p'-nitroazobenzene with peracetic acid. It is noteworthy that only one of the isomers was obtained. The other, β-p-bromo-p'-nitroazoxybenzene, was prepared from β-p-nitroazoxybenzene with bromine and a trace of iodine as a carrier. Its melting

Fig. 5. Structure of Isomers of Bromo and Nitro Derivatives of Azoxybenzene

point is 203°. Angeli and Alessandri (33) gave 199° as its melting point when made in the same way.

Other azoxy isomers that have been studied are the benzene-azoxy-p-phenols. It will be remembered that one of these, that with melting point 156°, was prepared by Bamberger (9) by the condensation of p-nitrosophenol and phenylhydroxylamine.

In 1914 Angeli (35) heated the acetyl derivative of benzeneazo-*p*-phenol with hydrogen peroxide and acetic acid and saponified the product. From it he obtained two benzeneazoxy-*p*-phenols, one with melting point 156°, identical with that of Bamberger, and another with melting point 107°. In a later research (36) he dealt with the constitution of these isomerides. They were found to be most easily separated by means of their benzoyl derivatives. When that of the isomer with melting point 107° was hydrolyzed it gave a compound with melting point 117°, showing that the value 107° found earlier was incorrect. Valori (37) shortly after

FIG. 6. CHANGES BROUGHT ABOUT BY BROMINATION OF α-BENZENEAZOXY-*p*-PHENOL AND REDUCTION OF THE PRODUCT

found it to be 117°, in agreement with Angeli's later value. Bigiavi and Sabatelli (38) in 1927 found it to be 118°. Angeli (36) suggested that of these two benzeneazoxy-*p*-phenols, that discovered by Bamberger and melting at 156°, should be given the α structure. In support of this view he brominated it, whereby a monobromo derivative was formed and later a dibromo derivative was obtained. A tribromo derivative could not be made by bromination. The dibromo derivative on reduction with zinc and acetic acid gave 2,6-dibromo-4-aminophenol, aniline and benzanilide. Since it is postulated that bromine enters the ring attached to a trivalent nitrogen and is, in fact, found in the ring containing the hydroxyl group, the conclusion follows that the hydroxyl group must be in this ring also. This establishes the

structure of the original benzeneazoxy-*p*-phenol as an α-compound. The relationship is shown in figure 6. It is interesting to report that a tribromo derivative of this compound could be obtained only by bromination of α-bromobenzeneazoxy-*p*-phenol as follows:

To the benzeneazoxy-*p*-phenol, *m.p.* 117°, must be given the β form. Figure 7 will illustrate this relationship and that of some of the derivatives of these compounds. Further, Valori (37) treated the ethyl ether of α-benzeneazoxy-*p*-phenol, *m.p.* 72°, with nitric acid and obtained benzeneazoxy-*m*-nitro-*p*-ethoxy-benzene, in this way further establishing the position of the trivalent nitrogen in the azoxy group. Thus considerable evidence has been adduced as to the structure of the two benzeneazoxy-*p*-phenols as given in figure 7.

Still another pair of isomers illustrates this phenomenon of isomerism. Bigiavi and Sabatelli (38) oxidized benzeneazo-*p*-toluene with peracetic acid and obtained two benzeneazoxy-*p*-toluenes. The α isomer

melts at 46°, the β isomer

obtained in smaller quantities melts at 65°. The former was accepted as the α compound since it did not react with bromine. The latter gave a *p*-bromo derivative and was therefore the β compound. Its constitution was established by reduction with

tin and hydrochloric acid to form p-bromoaniline and p-toluidine; and by reduction with aluminium amalgam to a hydrazo compound not isolated which, oxidized with mercuric oxide, gave p-bromobenzeneazo-p-toluene with a melting point of 153°, identical with that obtained from p-bromo aniline and nitrosotoluene by condensation. The β isomer, then, is the one containing the unsubstituted ring attached to a trivalent nitrogen. Fur-
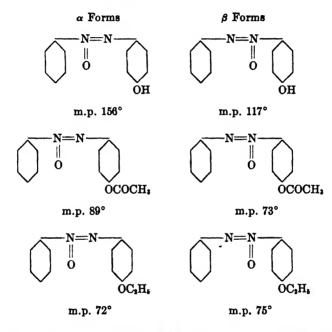
FIG. 7. α AND β FORMS OF BENZENEAZOXY-p-PHENOL AND SOME OF ITS DERIVATIVES
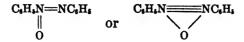
ther the β compound gave a p-nitro derivative, with melting point 163°, identical with the product obtained by the action of peracetic acid on p-nitrobenzeneazo-p-toluene.

The foregoing illustrations are well established examples of structural isomerism among azoxy compounds and constitute adequate experimental evidence of the existence of such isomers. The fundamental assumption on which their structure is based is that the oxygen of the azoxy group is attached to a pentavalent

nitrogen, the second nitrogen being trivalent as in azo compounds. In these latter compounds both bromine and nitric acid cause substitution in both rings; with the azoxy compounds in one ring only. The conclusion seems justified that the oxygen of the azoxy group exerts a protective action on the ring to which it is adjacent. On this assumption the isomers are classed as α or β. Further, such protective effect is not confined to the action of nitric acid and bromine only. In the case of the two benzene azoxy-p-toluenes already given (38) the α isomer is readily oxidized by chromic acid to the corresponding benzeneazoxy-p-benzoic acid, melting at 231°, while the β isomer is only slowly transformed into the corresponding compound melting at 241°. So great is the difference in the speed of the reactions that the β compound may be recovered in a pure condition after a mixture of the two is treated with chromic acid. A similar difference is observed in the case of the α and β hydroxy substituted benzenes as will be shown later.

II. MOLECULAR REFRACTION OF AZOXY COMPOUNDS

Further information concerning the structure of azoxy compounds has been adduced from a study of their absorption spectra, their molecular refractivity and diffraction, and from their molecular volumes.

Many investigators have studied the refractive power of both elements and compounds toward light of various wave lengths and, with a considerable degree of success, have been able to show a relationship between the structure of organic compounds and their molecular refractivity. The values to be ascribed to the atoms—the atomic refractivity—have been calculated from the data for molecular refractivity and in this way, also, the values for various groups and linkages have been determined. Bruhl, a distinguished investigator in this field, has carried out an extended series of investigations on organic nitrogen compounds. In two of his papers (39) he determined the constants for azoxybenzene and concluded that they are irreconcilable with the three-ring formula. He suggests that a diazonium-like structure would best represent it and that either

$$C_6H_5N=NC_6H_5 \qquad \text{or} \qquad C_6H_5N\!\!\equiv\!\!\!\equiv\!\!NC_6H_5$$

would represent the true structure, though he could not discriminate between them. No further study of the spectro-chemical behavior of azoxy compounds was made until 1928 when Auwers and Heimke (40) undertook a more extended investigation of these compounds. Auwers (41) had pointed out earlier that a three-membered carbon ring has a constant refractive increment and that the system A (figure 8) in ethylene oxide and related compounds is optically normal. The same is true for the three-membered system ethyleneimine B (figure 8) and its homologues, as pointed out by Markwald and Frobenius (42). Auwers (41) has further shown that the ring C (figure 8) in the dimethyl and diethyl esters of hydrazoisobutyric acid exerts no exalting
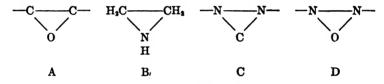
FIG. 8. CERTAIN THREE-MEMBERED RINGS

influence but, on the contrary, diminishes the molecular refractivity. Auwers and Heimke (43) conclude from these facts that there is no probability of the ring D (figure 8) increasing the molecular refractivity of a molecule. It is, however, a general characteristic of azoxy compounds that their molecular refractivities are high—always higher than that calculated from the atomic refractivities. These compounds, if of the ring structure, should give values for $E\Sigma_D^{20}$ approximately equal to that of hydrazobenzene and its derivatives. For these, however, this value is about 1.35, almost a complete unit below that of azoxybenzene.

Further, Auwers and Heimke examined the α and β forms of various azoxy compounds and found the differences in molecular refraction between them to be very small. They concluded that their isomerism is not due to a difference of structure like that between the old and the new formulation. Indeed they further

noted that recent research has more and more destroyed belief in three-membered rings. They however drew attention to the fact of their own experience that o,o'-azoxytoluene shows spectrochemical constants extraordinarily low. Further study is necessary for the explanation of this.

III. ABSORPTION SPECTRA

Studies in this field, particularly those of Hartley, have shown that substances of closely allied structure have absorption curves of similar form. By a comparison, then, of the absorption curve of a substance of unknown constitution with that of a known and related compound, valuable evidence regarding its structure may be obtained. If the molecular absorption curves for the two are similar, similarity of constitution may be assumed. There is much experimental evidence to support this conclusion. Absorption curves are plotted with frequency or wave length in Ångström units as abscissae and the log of molecular absorption as ordinates.

Szego (44) has studied the absorption spectra of a number of azoxy compounds in the ultra-violet part of the spectrum and has found that the curves for the isomeric α- and β-p-monobromo-azoxybenzenes and the similar pair of nitro isomers are very similar, but with the hydroxy substituted product, and with the p,p'-bromonitroazoxybenzene considerable differences are found. This is all the more noteworthy in the latter case since the entrance of halogen and nitro groups as substituents in the ring is generally without influence on the optical activity of compounds, as shown by Auwers (45). In general, however, Szego concluded that the results run parallel with the behavior of the two isomers as expected from their formulas as suggested by Angeli. All α forms show, besides the characteristic maximum for all azoxy compounds, a second band in the neighborhood of 2600 to 2500 Ångström units which the β forms lack. With α-2-methyl-5-hydroxyazoxybenzene three maxima are found compared with one in the β compound.

Szego (46) further carried out a comparison of the absorption curves of α and β isomers with that of the corresponding azo

compound. He found that the transformation of monobromo-
and mononitro-azobenzenes into the corresponding azoxy com-
pounds, by the entrance of oxygen, caused either no change in the
frequency of the band or a very small one. The bands in the azo
compounds were, in general, somewhat more sharply marked and
narrower. In the case of hydroxy substituted compounds, how-
ever, there is not so great a similarity. The α azoxy compound
and the similar azo body showed very similar curves with two
maxima, but the β had only one, or a second small one. He sug-
gested that, in the latter compound, the nearness of the hydroxyl
or amino group to the "active" oxygen of the azoxy group may
account for this irregularity. He also thought it quite possible
that there may be a second band in the α compound at a fre-
quency not yet examined. With symmetrically disubstituted
derivatives he found close agreement with the azo compound and,
in general, a slight displacement of the band toward the red from
azo to azoxy, with a more strongly marked band in the azoxy
compounds.
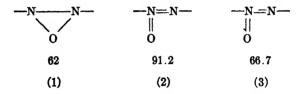
The similarity of the absorption curves of azoxy compounds
to those of the azo compounds suggests a similarity of structure.
To be of greatest value, however, it would be desirable to know
what influence, if any, three-membered rings containing nitrogen
have on absorption curves. It would seem that further work is
necessary before definite conclusions regarding the structure of
azoxy compounds can be arrived at from a study of absorption
spectra.

IV. PARACHOR OF AZOXY COMPOUNDS

Sugden's device (47) for comparing molecular volumes by the
use of a new constant called the "parachor" has excited much
interest and prompted considerable research. Through its
agency he has been able to determine atomic and structural con-
stants and from them to predict with considerable accuracy the
experimental values for a very large number of compounds.
Sugden's earlier work gave to the double bond between two atoms
the value of 23.2. In a considerable number of cases double bonds
showed a value of -1.6. Compounds with double bonds show-

ing this value all belong to the class in which the octet valence theory of Lewis (48) and Langmuir (49) leads to a modification of the usual structural formulas in which a valency bond is written for each pair of shared electrons.   This double bond, the so-called "semi-polar" double bond, has been found, according to Sugden (50), to be present in azoxy compounds.

From a study of the parachors of azoxybenzene the value for the group N_2O is calculated to be 64.7.   From a study of the parachors of o-azoxytoluene the value is found to be 70.6, clearly not a good agreement.   Below are given the calculated values for the group N_2O when represented in the three forms given:

—N————N—	—N==N—	—N=N—
\ /	‖	‖
O	O	O
62	91.2	66.7
(1)	(2)	(3)

The values as found by Sugden above would exclude form 2, that of Angeli, but would not distinguish between forms 1 and 3. The latter contains, as the octet theory would require, a semi-polar double bond as found in nitrobenzene.   Sugden concluded from electrochemical considerations and the fact that azoxy compounds are readily formed by oxidation of azocompounds, that form (3) represents the true linkage between the nitrogen and oxygen.   This formula, of course, would in no way invalidate Angeli's explanation of the isomerism of azoxy compounds.

More recent work by Mumford and Phillips (51) showed a closer agreement between the observed and calculated values of the parachor for azoxy compounds.   Using the newer values (52) found for the double bond between two nitrogen atoms and for nitrogen itself, and introducing the value for "strain constants" (53), the calculated value for the parachor for azoxybenzene became 447.5 and for o-azoxytoluene 527.5, compared with 444.7 and 528.6 found experimentally by Sugden, Reid and Wilkins (54).   These values strongly favor form 3 rather than the three-membered ring or the further form

$$-N \rightleftharpoons N-$$
$$\underset{O}{\overset{\|}{}}$$

suggested as a possibility by Mumford and Phillips (53), which gives a calculated value for azoxybenzene of 454 and for *o*-azoxytoluene of 534.0.

We may therefore conclude that the limited number of determined values for the parachors of azoxy compounds favors the formula suggested by Angeli for these compounds, modified by the introduction of the semi-polar double bond required by the octet theory.

V. WALLACH'S TRANSFORMATION

The well-known conversion of azoxybenzene into *p*-hydroxyazobenzene by the action of concentrated sulfuric acid was discovered by Wallach and Belli (55) and bears the name of the first of these authors. It is probably of very wide application and has been used to indicate the presence of an azoxy group. The general method is to heat the azoxy compound with the acid and pour the product into water, whereby there is obtained a colored compound soluble in alkali. Despite the simplicity of the method it seems not to have been studied as extensively as might be expected. The simple compound *p*-mononitroazoxybenzene has apparently not been treated in this way until recently. Bigelow and Linton (56) have found that this compound, if heated for a short time with concentrated sulfuric acid, gives an almost quantitative yield of *p*,*p*'-nitrohydroxyazobenzene and in a nearly pure condition. This compound was prepared originally by condensing *p*-nitrosophenol with *p*-nitroaniline, a process by no means simple. Nor has there been until recently a study of the effect of temperature upon the reaction which in the case studied, to be described later under bisazoxybenzenes, greatly modified the course of the reaction.

One might expect that, along with the *para* transformation one would find some of the *ortho* compound formed as well. Bamberger (57) has shown that in the treatment of azoxybenzene with sulfuric acid a small amount of this isomer is actually formed.

The same author (58) explains Wallach's transformation as follows:

$$C_6H_5N{=}NC_6H_5$$
$$\downarrow$$
$$C_6H_5N{=}N{=}C_6H_4\!\!\Big\langle$$
$$|$$
$$OH$$
$$\downarrow$$
$$C_6H_5N{=}NC_6H_4OH$$

in agreement with the hypothesis of Stieglitz (59). Here the transformation is due to the detachment and migration of a hydrogen atom with resulting free valence which effects the transposition.

Knipscheer (60) has found that at 200° p- and o-hydroxyazobenzenes are obtained by heating azoxybenzene with sulfuric acid, and on heating it with acetic anhydride the *ortho* compound is formed alone. This same *ortho* transformation is brought about, with some azoxy compounds at least, by the action of light.

The presence, the nature, and the position of substituents in the ring affect the nature of the transformation. Cusmano (61) found that o-aminoazoxybenzene with sulfuric acid gives phenylazimidobenzene

$$C_6H_4\!\!\left\langle\!\!\begin{array}{c}N\\|\\N\end{array}\!\!\right\rangle\!\!NC_6H_5$$

to the extent of 90 per cent yield, while at the same time a small quantity undergoes Wallach's transformation giving o-amino-p-hydroxyazobenzene.

Bamberger (62) found that sulfuric acid acting on his so-called iso-o-azoxybenzene, melting at 108–108.5°—one of the isomers—does not cause the Wallach transformation but acts as a reducing agent forming o-hydroxybenzeneazobenzene:

$$HOC_6H_4N{=}NC_6H_5$$

Angeli (63) thought to find in Wallach's transformation a means of separating the two α and β isomers from one another. Of the two p-bromo compounds

$$C_6H_5N{=}NC_6H_4Br \qquad \text{and} \qquad C_6H_5N{=}NC_6H_4Br$$
$$\parallel \qquad\qquad\qquad\qquad\qquad\qquad \parallel$$
$$O \qquad\qquad\qquad\qquad\qquad\qquad O$$
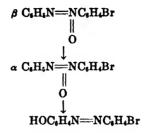
he expected the α form to undergo the transformation, the β form to be unattacked. In fact both of them give the same final product

$$HOC_6H_4N{=}NC_6H_4Br$$

when heated with sulfuric acid. This, according to Angeli, makes it very probable that the hot acid transforms the β into the α form which then undergoes transformation into the hydroxyazo compound. He found support for this view in the fact that nitric acid (density 1.45) acting on the two p-nitroazoxybenzenes
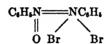
$$C_6H_5N{=}NC_6H_4NO_2 \qquad \text{and} \qquad C_6H_5N{=}NC_6H_4NO_2$$
$$\parallel \qquad\qquad\qquad\qquad\qquad\qquad \parallel$$
$$O \qquad\qquad\qquad\qquad\qquad\qquad O$$

α-p-Nitroazoxybenzene $\qquad\qquad$ β-p-Nitroazoxybenzene

nitrated only the β form, the $\begin{array}{c}{-}N{=}\\\parallel\\O\end{array}$ group protecting the benzene

ring to which it is attached from the action of the acid. When, however stronger nitric acid (density 1.52) was used, both forms gave the same polynitro derivatives. To determine whether this action was due to the oxidizing nature of the acid he substituted chromic acid for nitric on the α and β forms of both p-bromo- and p-nitro-azoxybenzene. Both the α and β forms of the bromo compound and the α form of the nitro compound were unchanged by chromic acid but the β form of the latter was changed into the corresponding α form. He concluded, then, that when the β form was treated with sulfuric acid the following transposition took place:

$$\beta\ C_6H_5N=NC_6H_4Br$$
$$\|$$
$$O$$
$$\downarrow$$
$$\alpha\ C_6H_5N=NC_6H_4Br$$
$$\|$$
$$O$$
$$\downarrow$$
$$HOC_6H_4N=NC_6H_4Br$$

Possibly the β form took up an atom of oxygen and lost the one already present. That the chromic acid acted as an oxidizing agent was suggested by its reduction to a chromic condition. Further, that such an oxidation is possible was suggested by the discovery of Wohl (64) that azoxybenzene is capable of forming an unstable dibromo addition product, perhaps of the formula

$$C_6H_5N=\!=\!=NC_6H_5$$
$$\|\ \diagup\ \diagdown$$
$$O\ Br\ \ \ \ Br$$

VI. BISAZOXY COMPOUNDS

Useful information concerning the possibility of isomerism of azoxy compounds may be found in the study of those bodies containing more than one azoxy groups. With this in view Angeli (65) treated bisazobenzene, previously prepared by Mills (65), with hydrogen peroxide and glacial acetic acid and obtained a yellow substance melting at 155°. It is apparent that there should be three possible isomeric forms of bisazoxybenzene (see figure 9).

The compound obtained by Angeli was very pure. To determine which of these three formulas should be ascribed to his compound he treated it with bromine and obtained a dibromo substitution product. Formula 1 should give no bromo derivative; formula 2 should give a monobromo derivative; and formula 3 shall give a disubstitution product. He therefore concluded that formula 3 should be ascribed to his compound. He further found that on reduction it gave the original bisazobenzene with which he started, showing that the reaction is reversible.

This bisazoxybenzene was treated with concentrated sulfuric

acid at 100° and was easily transformed into two products, both soluble in alkali with a red color.   He assumed that they were the compounds represented in figure 10.   One of these was so insoluble in all solvents as to make it impossible to characterize it, but the constitution of the other was established by synthesis. Aminoazobenzene was diazotized in the presence of hydrochloric acid and the product treated with phenol.   The product was found identical with the new compound which must therefore have the formula 1 (figure 10).   This formula he assigned to it.

(1)
$$C_6H_5N \!=\! NC_6H_4N \!=\! NC_6H_5 \quad m.p \; 168°$$
$$\overset{\parallel}{O} \qquad\qquad \overset{\parallel}{O}$$

(2)
$$C_6H_5N \!=\! NC_6H_4N \!=\! NC_6H_5 \quad m.p. \; 148°$$
$$\overset{\parallel}{O} \qquad \overset{\parallel}{O}$$

(3)
$$C_6H_5N \!=\! NC_6H_4N \!=\! NC_6H_5 \quad m.p. \; 155°$$
$$\quad\overset{\parallel}{O} \;\; \overset{\parallel}{O}$$

FIG. 9. THE THREE POSSIBLE ISOMERIC FORMS OF BISAZOXYBENZENE

(1)
$$C_6H_5N \!=\! NC_6H_4N \!=\! NC_6H_4OH$$

(2)
$$HOC_6H_4N \!=\! NC_6H_4N \!=\! NC_6H_4OH$$

FIG. 10. SUBSTANCES FORMED BY THE ACTION OF CONCENTRATED SULFURIC ACID ON BISAZOXYBENZENE

The original bisazoxybenzene, melting at 155°, when treated with sulfuric acid at 0°, gave a very different product from that obtained at 100°.   On prolonged treatment the compound disappeared and other products were formed—one insoluble in alkalies, orange-yellow in color; and a second, red in color, and soluble in alkalies with a reddish brown color, therefore a hydroxy derivative.   The first product, that insoluble in alkalies, with proper solvents was separated into two compounds with melting points of 168° and 148° respectively.   The yellow compound, with melting point 168°, when treated with sulfuric acid

at 100° gave the same monohydroxybisazobenzene given by Angeli's isomer (formula 3 in figure 9); on reduction with aluminium amalgam it gave the original bisazobenzene of Mills. It was unattacked by bromine and therefore Angeli concluded it was a bisazoxybenzene and was represented by formula 1 in figure 9.

The orange compound, melting at 148°, was obtained in small amount. It was unattacked by sulfuric acid at 0° and the author, noting the difficulties in its characterization, ascribed to it formula 2 (figure 9).

It is notable in this study of the bisazoxy isomers that peracetic acid, acting on bisazobenzene, gives only one of the three possible isomers—the one in which the oxygen atoms are attached to the nitrogens distant from the end nuclei. This is all the more notable since simple azo compounds under the action of peracetic acid give, in general, both isomers. The action of sulfuric acid is also unique in transforming one isomer into another. Though rare it has its analogy, as we have already seen, in the action of chromic acid on β-p-nitroazoxybenzene.

VII. TRISAZOXYBENZENES

The successful preparation of the bisazoxybenzenes was coincident with the preparation of trisazoxy compounds (65). Angeli found in azoxybisazobenzene

$$C_6H_5N=NC_6H_4N=NC_6H_4N=NC_6H_5$$
$$\|$$
$$O$$

a starting point for their synthesis. This compound was prepared by Angeli by reducing p-nitroazobenzene with sodium alcoholate. It melted at 215°. Angeli apparently overlooked the fact that Borsch (67) had already made this compound by the action of alkali on quinonoximebenzoylphenylhydrazone and had found that it melted at 218°. Borsch had also prepared it by the action of sodium methoxide on p-nitroazobenzene, a method analogous to that used by Angeli. The agreement in melting point is not too satisfactory.

This compound was treated with peracetic acid and after boiling for several days there was obtained a golden yellow compound which melted at 230°. Four trisazoxybenzenes are possible (see figure 11).

Of these the one represented by formula 1 should be unaffected by bromine; those represented by formulas 2 and 3 should give monosubstitution products; while that represented by formula 4 should give a dibromo substitution product. The compound melting at 230° was treated with bromine in the cold with a trace of iron dust. It gave a dibromo substitution product and Angeli concluded, therefore, that its structure is represented by formula

(1)
$$C_6H_5N{=}NC_6H_4N{=}NC_6H_4N{=}NC_6H_5$$
$$\underset{O}{\|} \qquad \underset{O}{\|} \qquad \underset{O}{\|}$$

(2)
$$C_6H_5N{=}NC_6H_4N{=}NC_6H_4N{=}NC_6H_5$$
$$\underset{O}{\|} \qquad \underset{O}{\|} \qquad \underset{O}{\|}$$

(3)
$$C_6H_5N{=}NC_6H_4N{=}NC_6H_4N{=}NC_6H_5$$
$$\qquad \underset{O}{\|} \quad \underset{O}{\|} \qquad \underset{O}{\|}$$

(4)
$$C_6H_5N{=}NC_6H_4N{=}NC_6H_4N{=}NC_6H_5$$
$$\qquad \underset{O}{\|} \quad \underset{O}{\|} \qquad \underset{O}{\|}$$
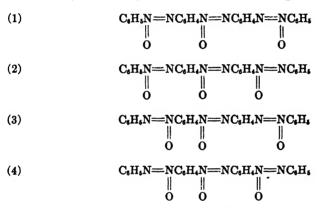
FIG. 11. THE FOUR POSSIBLE ISOMERIC TRISAZOXYBENZENES

4 (figure 11). Later Valori (68) prepared this compound by the oxidation of p-aminoazobenzene with peracetic acid.

Passerini (69) in an endeavor to make another of these trisazoxybenzenes started with the acetyl derivative of p-aminoazobenzene whose melting point, curiously, he reported as 157° though the literature gives it as 146°. This latter value Bigelow and McNevin (70) have corroborated by preparing the compound from pure aminoazobenzene and acetyl chloride. Passerini assumed that the acetyl group protected the amino group from oxidation and that on oxidation with peracetic acid he obtained the two corresponding acetylaminoazoxybenzenes. These he hydrolyzed with sodium hydroxide to obtain the corresponding

aminoazoxybenzenes. These were further oxidized with peracetic acid to give the trisazoxybenzene of Angeli, melting at 230°, and another melting at 223° to which he assigned the formula 4 (figure 11). In figure 12 is illustrated in tabular form his

$$C_6H_5N=NC_6H_4NHCOCH_3$$

m.p. 157° (Passerini)
m.p. 146° (Literature)

$$H_2O_2$$

α $C_6H_5N=NC_6H_4NHCOCH_3$
$\parallel$
O

m.p. 166° (Passerini)
m.p. 151° (Bigiavi)

| NaOH

α $C_6H_5N=NC_6H_4NH_2$
$\parallel$
O

m.p. 134.6° (Passerini)
m.p. 136° (Bigiavi)
m.p. 138.5° (Schmidt)

$$H_2O_2$$

$C_6H_5N=NC_6H_4N=NC_6H_4N=NC_6H_5$
$\parallel$ $\parallel$ $\parallel$
O O O

m.p. 223° (Passerini)
m.p. 242° (Bigelow and McNevin)

β $C_6H_5N=NC_6H_4NHCOCH_3$
$\parallel$
O

m.p. 142–3° (Passerini)
m.p. 172° (Bigiavi)

NaOH

β $C_6H_5N=NC_6H_4NH_2$
$\parallel$
O

m.p. 114° (Passerini)
m.p. 133° (Bigiavi)

$$H_2O_2$$

$C_6H_5N=NC_6H_4N=NC_6H_4N=NC_6H_5$
$\parallel$ $\parallel$ $\parallel$
O O O

m.p. 230° (Angeli)

FIG. 12. SYNTHESIS OF TRISAZOXYBENZENES

process of synthesis with the melting points for the compounds as found by him and others. Very evidently he was working with impure products.
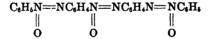
Bigiavi (71) has denied the accuracy of Passerini's primary assumption that the acetyl group protects the amino group from

oxidation and has shown that, in fact, the amino group is partially oxidized to a nitro group, thus forming the two isomeric nitroazoxybenzenes as well as the isomeric *p*-acetylaminoazoxybenzenes. These facts would account for the differences in melting points of the various compounds formed and throw doubt on the accuracy of the melting point of the compound to which Passerini gave formula 1 (figure 11).

Further Bigelow and McNevin (70), by reducing *p*-nitroazoxybenzene with sodium arsenite, obtained as chief product of the reaction the new compound azobisazoxybenzene

$$C_6H_5N{=}NC_6H_4N{=}NC_6H_4N{=}NC_6H_5$$
$$\|\qquad\qquad\qquad\qquad\qquad\|$$
$$O\qquad\qquad\qquad\qquad\qquad O$$

which was oxidized with peracetic acid and gave a golden yellow compound melting at 242°. Its analysis and properties showed it to be the trisazoxybenzene having the structure of formula 1 in figure 11 which Passerini ascribed to his compound with melting point 223°. By means of a new synthesis we are now seeking to further establish the structure of this compound.

Of the corresponding *o*-trisazoxybenzenes, Cusmano (72) has prepared one in small quantity by the action of a trace of sodium hydroxide on *o*-hydroxylaminoazoxybenzene. To it he ascribed the formula

$$C_6H_5N{=}NC_6H_4N{=}NC_6H_4N{=}NC_6H_5$$
$$\|\qquad\qquad\|\qquad\qquad\|$$
$$O\qquad\qquad O\qquad\qquad O$$

Charrier and Crippa (73) treated *o*-aminoazobenzene with peracetic acid for twenty-eight days and obtained three products, one of which, melting at 278°, they believed to be an *o*-trisazoxybenzene. It had the molecular proportions of such a compound and on reduction gave aniline and *o*-phenylenediamine, but they were not able to characterize it with certainty.

VIII. CYCLIC AZOXY COMPOUNDS

Only a few cyclic azoxy compounds have been prepared.

Täuber (74) succeeded in preparing a cyclic azoxy compound by reducing *o*-dinitrodiphenyl in an alkaline medium. Besides other

compounds he obtained one to which he ascribed the structural formula of an azoxy compound

$$N\!-\!\!-\!N \diagdown\!\!\diagup O$$

which today would be written

$$N\!=\!\!=\!N \quad \overset{\shortparallel}{O}$$

Ullman and Dieterle (75) in 1904 prepared the same compound and called it diphenazonoxyd. It might, perhaps, be better called cyclic *o,o'*-azoxydiphenyl. They prepared, also, the dimethyl derivative, the methyl groups being *meta* to nitrogen atoms.

Duval (76) and King (77) succeeded in preparing 4,4'-diamino-2,2'-azoxydiphenylmethane by reducing 2,2'-dinitro-4,4'-diaminodiphenylmethane with sodium sulfide in an alcoholic solution. Duval, also, in 1910 (78) prepared 2,2'-azoxydiphenylmethane-4,4'-dicarboxylic acid.

Arndt (79) prepared a substance which he considered a cyclic azoxy compound by boiling *o*-nitrophenylguanidine with dilute alkali. Ring closure was shown to occur through the elimination of water, the participating groups being the nitro and amino groups. The compound formed was 1,2,4-benztriazine-1-oxide which he considered an azoxy compound. Arndt and Rosenau (80) in 1917 were successful in preparing several cyclic azoxy compounds of this sort and found that they were capable of reduction to azo and hydrazo compounds and, further, that they could be regenerated from the azo compound by oxidation with hydrogen peroxide.

More recently Hussey, Marvel and Hager (81) have prepared ethyl-2,2'-azoxydiphenylmethane-4,4'-dicarboxylic acid by an interesting synthesis from benzophenone. This compound, having the structural formula

should be optically active and might, then, be capable of resolution into its isomers. The attempted resolution was not conclusive and the work is being continued.

IX. OXIDATION OF AZOXY COMPOUNDS

In general azoxy compounds are extremely resistant to oxidation. The special action of nitric, chromic and sulfuric acids has already been discussed. In these cases the action is not one of oxidation. With the hydroxyl-containing azoxy compounds, however, the situation is unique. They are oxidized with a considerable degree of ease with a breaking down of the ring.

Bamberger (82) found that both the *ortho* and isohydroxyazoxybenzenes which have already been described were oxidized to isodiazotates by alkaline permanganate. So also was *p*-hydroxyazoxybenzene.

Angeli (83) studied the two *p*-hydroxyazoxybenzenes

When the α form was heated with 2 per cent potassium permanganate in alkaline solution, oxidation began at once; Angeli noted

the odor of both nitrosobenzene and nitrobenzene and obtained the isodiazotate

$$C_6H_5N\!=\!N\!-\!OH$$

The β form was much more resistant to oxidation under the same conditions. Only on standing over night did he note reduction of the permanganate. He found in this fact a method for the separation of the β form in a condition free from the α form. Further, a study of the *o*-hydroxyazoxybenzenes of Bamberger proved that these show the same differences in ease of oxidation.

Angeli also treated *p,p'*-azoxyphenol with 2 per cent permanganate in alkaline solution until a permanent color remained. The filtrate from this mixture gave no color with β-naphthol. No diazo compound was formed, but oxalic acid was found in quantity.

Bigiavi and Poggi (84) oxidized benzeneazo-*p*-cresol

with peracetic acid and obtained the two isomeric α- and β-azoxy compounds

In alkaline permanganate solution these isomers showed the same general action as the corresponding α- and β-*p*-hydroxyazoxybenzenes. In this case the β form was oxidized to so slight a degree that the normal diazo compound obtained by acidifying the isodiazo compound gives but a feeble red color with β-naphthol.

This action of permanganate on the α and β hydroxyl containing azoxybenzenes is comparable with that observed in the action of bromine or nitric acid upon the isomeric monosubstituted compounds—an action already described—and illustrates again

the protective action of the oxygen of the azoxy group upon the nucleus to which it is adjacent.

X. NITRATION OF AZOXY COMPOUNDS

The usual method for preparing a substituted azoxy compound is to reduce, with a suitable reducing agent, the corresponding substituted benzene containing, in addition, a nitro group. Thus p-chloronitrobenzene on reduction with alcoholic potassium hydroxide (85) gives p,p'-dichloroazoxybenzene. The nitro group may be replaced by a nitroso or hydroxylamine group. Indeed there is a variety of methods, but the most general is to start with a nitro compound having the substituted groups desired and to transform it into an azoxy compound.

When more than one nitro group is present in the benzene compound reduced, then a nitrated azoxy compound is produced. Thus m-dinitrobenzene gives, on reduction with sodium methoxide, m,m'-dinitroazoxybenzene.

Similarly the *para* compound can be made (87) by the action of sodium hydroxide on p-dinitrobenzene.

A further method for the preparation of nitrated azoxybenzene is that of direct nitration. It has already been pointed out that concentrated nitric acid will attack only one of the rings in azoxy compounds. The simplest compounds so formed are the mononitroazoxybenzenes discovered by Zinin (88). Both the *para* and *ortho* isomers are formed by treating azoxybenzene with concentrated nitric acid, keeping the mixture cooled. These isomers, of which the *para* is much the more abundant, are separated from one another with some difficulty.

The nitration of azoxybenzene with fuming nitric acid is attended with serious difficulties in separating the various products from one another, as various investigators have found.

From 2,4,4'-trinitroazobenzene Werner and Stiasny (89) succeeded in making 2,4,4'-trinitroazoxybenzene by treating the

azo compound with fuming nitric acid and chromic acid; by similar treatment of 2,4,3'-trinitroazobenzene, they prepared 2,4,3'-trinitroazoxybenzene. This method is unusual and is of special interest because of its analogy to the method of preparing azoxy compounds by oxidizing azo compounds with peracetic acid. These two trinitroazoxybenzenes were earlier made by Klinger and Zuurdeeg (90) in an interesting study of the nitration of azo and azoxy compounds.

The 3,5,3',5'-tetranitroazoxybenzene

$$\text{O}_2\text{N} \underset{\text{NO}_2}{\overset{-\text{N}=\text{N}---}{\underset{\text{O}}{\parallel}}} \text{O}_2\text{N} \quad \text{NO}_2$$

is made by reducing 1,3,5-trinitrobenzene with a concentrated sodium hydroxide solution (91).

The following section describes a further method of nitration of azoxy compounds applicable in the case of those containing a hydroxyl group.

XI. NITROUS ACID AND AZOXYPHENOLS

The protective action of the oxygen atom of the azoxy group toward the adjacent nucleus is shown also in the action of cold, dilute nitrous acid upon azoxyphenols. In this case again only the α isomer is attacked, the action being a particularly interesting one. Here the action is not one of oxidation but of nitration. For this action the presence of the hydroxyl group is a prerequisite.

Angeli, Bigiavi and Carrara (92) have shown that nitrous acid acts on the grouping

$$\text{HOC}_6\text{H}_4\text{N}=\text{N}---$$
$$\overset{\parallel}{\text{O}}$$

giving rise to a nitro derivative with the nitro group in the *ortho* position to the hydroxyl, while it is without action on the grouping

$$\text{HOC}_6\text{H}_4\text{N}=\text{N}--$$
$$\overset{\parallel}{\text{O}}$$

In harmony with these facts it has been found that azoxybenzene and β-p-azoxyphenol are not changed when excess of sodium nitrite is gradually added to their glacial acetic acid solution, while α-p-azoxyphenol and p,p'-dihydroxyazoxybenzene under the same conditions readily yield nitro derivatives.

Thus α-p-hydroxyazoxybenzene

$$C_6H_5N{=}NC_6H_4OH$$
$$\overset{\|}{O}$$

gives α-benzeneazoxy-p-hydroxy-m-nitrobenzene

the corresponding β compound being unchanged.

The dihydroxy compound, p,p'-dihydroxyazoxybenzene

$$HOC_6H_4N{=}NC_6H_4OH$$
$$\overset{\|}{O}$$

under the action of nitrous acid gives the mononitro derivative

though p-dihydroxyazobenzene

$$HOC_6H_4N{=}NC_6H_4OH$$

under the same conditions gives 3,3'-dinitro-4,4'-dihydroxyazo-benzene

So also with benzeneazoxy-*p*-cresol

the corresponding benzeneazoxy-*o*-nitro-*p*-cresol

is obtained.

XII. THE ACTION OF LIGHT ON AZOXY COMPOUNDS

Wacker (93) found that when filter paper was impregnated with α-azoxynaphthalene dissolved in alcohol and placed in direct sunlight, it was colored red in a short time. The same was true also of the solution. He claimed, too, that azoxybenzene showed the same property though the action was slower. Knipscheer (60) stated that light acting on azoxybenzene gives *o*-hydroxy-azoxybenzene. He obtained a yield of about 10 per cent by exposing sheets of filter paper impregnated with this compound to sunlight for five weeks. He believed that the action took place when the solid was exposed to light.

Baudisch and Fürst (94) noted the probable change, in sunlight, of α-azoxynaphthalene into α-hydroxyazonaphthalene. They then subjected the α-azoxynaphthalene in methyl alcohol to sunlight. After an hour brownish red needles were obtained which proved to be pure α-hydroxyazonaphthalene. Corresponding with this work of Baudisch and Fürst may be mentioned that of Cumming and Steel (16) which they believed gave evidence for the existence of two isomeric *o*-azoxynaphthalenes and of Cumming and Ferrier (18) who found that light on α-azoxy-naphthalene gives the same hydroxyazonaphthalene found by Baudisch and Fürst above. Cumming and Ferrier studied the action of ultra-violet light on azoxybenzene and obtained a 30 per cent yield of *o*-hydroxyazobenzene.

Bigiavi and Sabatelli (95) found that the action of light on

α-benzeneazoxy-p-toluene, melting at 46°, gave a red substance, $m.p.$ 112–114°, which they regarded as probably the o-hydroxy compound.

The photochemical action of azoxy compounds has been little studied, though it is clear that it is a field offering many opportunities for research. In particular it is desirable to determine with certainty whether or not the action of light causes isomerism. It would rather seem, at present, that the evidence does not strongly support belief in this hypothesis.

XIII. THE GRIGNARD REAGENT AND AZOXY COMPOUNDS

Concerning the action of the Grignard reagent on azoxy compounds very little seems to have been published. Cumming and Ferrier (96) attempted to determine the best conditions for the interaction between ethylmagnesium iodide and azoxybenzene and found that the reaction products were azobenzene and a brown oil, which has the odor of ethylaniline but could not be identified as such.

XIV. PREPARATION OF AZOXY COMPOUNDS

Although a discussion of the preparation of azoxy compounds may not be pertinent to the title of this paper the subject is briefly introduced here because of its importance and more particularly because it throws light on the reduction of these compounds.

According to Haber's rule (97) the direct line of reduction of aromatic nitro compounds is represented as follows:

$$C_6H_5NO_2 \rightarrow C_6H_5NO \rightarrow C_6H_5NHOH \rightarrow C_6H_5NH_2$$

and azoxy compounds are formed by condensation of nitroso and hydroxylamine intermediates. However true the primary assumption may be, there is much evidence to support the view that azoxy compounds are formed by the above condensation.

This condensation takes place best in alkaline solution. Some have held the view (98) that, in acid solution, nitroso compounds are reduced with infinite velocity, precluding condensation. This view, however, is untenable since Flürscheim (99) has

shown that in numerous cases reduction may give rise to azoxy compounds even in the presence of strong mineral acids. Flür- scheim and Simon (100) showed that both condensation and re- duction of nitroso and hydroxylamine derivatives take place at measurable rates whether the solution be acid, neutral or alkaline. A priori, then, neither reduction of the intermediate compounds to amines nor condensation to azoxy compounds is excluded. It is possible, however, to vary the velocity of these reactions within wide limits so that one may be retarded to make another the primary or sole product.

Flürscheim and Simon considered that the primary determi- nant of the course of the reaction is the valence of the nitrogen in the hydroxylamine derivative formed. If trivalent, con- densation is favored; if pentavalent, condensation will not take place and the amine will be formed. In an acid solution the nitrogen of the hydroxylamine will be pentavalent and so reduc- tion to the amine will be the chief course of the reaction. Still other factors influence the course of the reaction, such as strength of the hydroxylamine base, the concentration of acid, the nature of the solvent, the relative concentration of nitro compound and reducing agent, the strength of reducing agent, the temperature, and the constitution of the nitro compound.

With respect to his last influence it is of interest to note the effect of negative substituents on the course of the reaction. Nitrobenzene gives no perceptible yield of azoxybenzene when reduced with stannous chloride even in the absence of acid; p- nitrobenzaldehyde, with its moderately negative aldehyde group, gives a small yield of p-azoxybenzaldehyde; but 3,5-dichloro-4- bromonitrobenzene, being still more negatively substituted, gives an almost quantitative yield of azoxy compound under similar conditions.
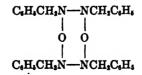
Though it is possible to reduce some nitro compounds to azoxy derivatives in acid solution, most of the methods for the prepara- tion of the latter require an alkaline medium. These methods may be classified as those of condensation, oxidation and reduc- tion and as such will be discussed.

Condensation

Merz and Coray (101) treated a mixture of aniline and nitrobenzene with solid potassium hydroxide. A vigorous reaction took place with the evolution of a combustible gas and the formation of a resinous mass from which azobenzene was obtained. The authors believed that azoxybenzene was also present. We have already discussed the work of Angeli in preparing azoxybenzene from these compounds through the agency of metallic sodium.

Fischer and Wacker (102) found that *p*-nitrosodimethylaniline condensed with phenylhydroxylamine in absolute alcohol to give *p*-azoxydimethylaniline. A similar reaction took place in potassium hydroxide solution. Using the hydrochloride of phenylhydroxylamine, however, the reaction product was dimethylphenylene diamine.

Bamberger and Renauld (4), as already stated, found that nitrosobenzene and phenylhydroxylamine condense to give azoxybenzene without by-products. In the same manner, using substituted phenylhydroxylamines and nitrosobenzene, they succeeded in preparing *m*-azoxytoluene, *p*-azoxytoluene, *p*-dichloroazoxybenzene, *m*-dibromoazoxybenzene. and *p*-dibromoazoxybenzene, all symmetrical azoxy compounds. In each case azoxybenzene was formed as well. They concluded that the nitrosobenzene oxidized the hydroxylamine derivative to an azoxy compound and was itself reduced to azoxybenzene. It is interesting to note that by treating nitrosobenzene with benzylhydroxylamine they obtained bisazoxybenzyl, a compound with the proportions of an azoxy compound but with twice its molecular weight. They gave to it the structural formula

$$C_6H_5CH_2N\!-\!NCH_2C_6H_5$$
$$|\quad\ |$$
$$O\quad O$$
$$|\quad\ |$$
$$C_6H_5CH_2N\!-\!NCH_2C_6H_5$$

Later Bamberger and Bernays (103) by condensing phenylhydroxylamine and *p*-nitrosophenol to form *p*-monohydroxyazoxy-

benzene and azoxybenzene showed, in fact, that mixed azoxy compounds could be made by a process of condensation. The reaction is, however, not a simple one, other products being formed as well.

Brand and Stohr (104) studied the effect of neutral, acid and alkaline media on the condensation of o-nitrosoacetanilide with o-hydroxyaminoacetanilide, dilute alcohol being the solvent. They found that the reaction was most complete in alkaline solution, less in acid and least in neutral.

Oxidation

Petriew (105) prepared azoxybenzene by oxidation of azo benzene with chromic acid in an acetic acid solution in a closed tube at 150° to 250°. With nitric acid of specific gravity 1.54 azobenzene gave trinitroazoxybenzene, the azo group being oxidized and the rings nitrated as well.

Hantzsch and Lehmann (106) oxidized bisdiazoacetic acid to bisazoxyacetic acid, to which they gave the structure

$$HOOCCH \underset{N-N}{\overset{N-N}{\diamond}} CHCOOH$$

From this they obtained on heating bisazoxymethane, with the structure

$$H_2C \underset{N-N}{\overset{N-N}{\diamond}} CH_2$$

Bamberger and Tschirner (107) obtained azoxybenzene with other products by the oxidation of aniline with potassium permanganate in the presence of formaldehyde and sulfuric acid.

Bamberger and Hübner (108) oxidized *m*-nitroaniline with mono-persulfuric acid and obtained *m*-dinitroazoxybenzene and, in addition, *m*-nitronitrosobenzene and *m*-dinitrobenzene. From *p*-nitroaniline with the same reagent they obtained the corresponding azoxy derivative.

Werner and Stiasny (109) oxidized *p*-dinitroazobenzene to *p*-dinitroazoxybenzene by using fuming nitric acid at room temperature.

Prud'homme (110) oxidized aniline to azoxybenzene with hydrogen peroxide and obtained azoxybenzene. We have already described Angeli's method for the preparation of azoxy compounds by treating azo bodies with this reagent. Glacial acetic acid is the usual solvent used.

In alkaline solution Limprecht (111) oxidized *m*- and *p*-amino-benzenesulfonic acids with potassium permanganate and obtained the corresponding azoxybenzenemonosulfonic acids. With these, azo compounds were formed as well.

Bamberger (112) found that phenylhydroxylamine was oxidized to azoxybenzene when heated in the air on a steam bath. Wacker (113) found that *α*-naphthylhydroxylamine under the same conditions was oxidized to *α*-azoxynaphthalene.

Reduction

Flürscheim's assumption (99) that condensation of hydroxylamine derivatives and nitroso compounds to form azoxy compounds takes place best when the hydroxylamine derivative is in the free state, led him to the conclusion that the presence of substituting groups in the ring would favor this condensation even in the presence of acids. To determine the accuracy of this assumption he reduced a number of such substituted compounds in acid solution. *m*-Dinitrobenzene with stannous chloride and hydrochloric acid gas in absolute alcohol gave *m*-dinitroazoxybenzene in fair yield. Symmetrical trinitrobenzene, under similar conditions, gave a better yield of tetranitroazoxybenzene while *m*-dichloro-*p*-bromonitroazoxybenzene gave an almost quantitative yield of dibromotetrachloroazoxybenzene.

An alkaline medium is, however, the most effective in which to

prepare azoxy compounds. Many reducing agents are effective, among which sodium and potassium methoxides and ethoxides have been much used. Klinger and Pitschke (114) reduced *m*-dinitrobenzene to *m*-dinitroazoxybenzene with sodium methoxide. Lachmann (115) prepared azoxybenzene from nitrobenzene by this reagent and Heumann (116) used sodium ethoxide in reducing *p*-chloronitrobenzene to *p*-dichloroazoxybenzene.

deBruyn and Blanksma (117) treated *m*-dinitrobenzene with sodium acid sulfide in alcohol and obtained some *m*-dinitroazoxybenzene and *m*-nitroaniline. Blanksma (118) with sodium disulfide reduced *m*-dinitrobenzene to the corresponding azoxy derivative in almost quantitative yield. With the *para* compound an azo derivative was the principal product.

Meldola and Andrews (119) reduced *m*-nitroaniline with stannous chloride and sodium hydroxide and obtained *m*-diaminoazoxybenzene and other products.

With concentrated sodium hydroxide deBruyn (120) reduced *p*-dinitrobenzene to *p*-dinitroazoxybenzene and 1,3,5-trinitrobenzene to 3,5,3′,5′-tetranitroazoxybenzene. With ferrous sulfate and sodium hydroxide Alway and Bonner (121) reduced *m*-nitrobenzaldehyde to *m*-azoxybenzaldehyde. Using 60 per cent sodium hydroxide and iron pyrites (122) the Bayer Co. found that, from nitrobenzene, a yield of 90 per cent azoxybenzene could be obtained. With zinc dust and sodium hydroxide Guitermann (123) reduced *o*-nitrotoluene in an alcoholic solution to azoxytoluene.

Using sodium amalgam in a small amount of alcohol Melms (124) found that nitrotoluene gave azoxytoluene. Evans and Fry (125) used magnesium amalgam as a reducing agent in alcohol. From nitrobenzene they obtained azoxybenzene in 90 per cent yield, and from *o*- and *p*-nitrotoluenes they obtained the corresponding azoxy compounds. Using methyl alcohol instead of ethyl alcohol nitrobenzene gave azobenzene, *m*-dinitrobenzene gave dinitroazoxybenzene and a small amount of the corresponding azo compound, while the *o*- and *p*-nitrotoluenes gave both the azo and azoxy compounds but principally the former.

Sodium arsenite has been used to a limited extent as a reducing

agent for nitro compounds. Loesner (126) prepared azoxybenzene from nitrobenzene with this reagent in a strong alkaline solution. Vorländer (127) in a study of liquid crystals, a phenomenon exhibited by a variety of azoxy compounds, prepared azoxybenzalacetophenone from nitrobenzalacetophenone with this reagent. Bigelow and Philp (128) found that sodium arsenite acting on *m*-nitrobenzaldehyde gave *m*-azoxybenzyl alcohol and *m*-azoxybenzoic acid. Evidently the Cannizaro reaction accompanied the reduction of the nitro group.

Electrochemical reduction of nitro compounds in acid solution gives, in general, amino compounds. If the acid used is sulfuric, or especially acetic, aminophenols are formed. Elbs (129) electrolyzed nitrobenzene in a strong sodium hydroxide solution between platinum and iron electrodes, using a current density of 8.33 amperes per square decimeter. He obtained a 60 per cent yield of hydrazobenzene. Using sulfuric acid instead of sodium hydroxide, benzidine was the principal product and with it a small amount of azoxybenzene. Hausmann (130) showed that the reduction of *o*-nitroanisol in alkaline solution gave azoxy and hydrazo compounds. Lob (131) using a platinum anode and a cathode of nickel in 2 to 4 per cent aqueous alkali and with a current density of 5 to 7 amperes per square decimeter obtained a yield of 90 per cent azoxybenzene from nitrobenzene. Elbs, however, (132) found that *o*- and *p*-nitrophenol, even in alkaline solution, gave the corresponding amines. Phenol ethers were reduced to azoxy compounds. Further, Häusermann and Schmidt (133) found that several nitrophenyl ethers gave analogous azoxy compounds as a result of electrolytic reduction. Lob (134) reduced a 5 per cent solution of *o*-nitrobenzoic acid in a 5 per cent sodium hydroxide solution at the platinum cathode of a divided cell with a current density of 4 amperes. The yield was 50 per cent azoxybenzoic acid with 5 to 10 per cent hydrazobenzoic acid. Brand and Stohr (135) reduced *o*-nitroacetanilide electrolytically in the presence of sodium acetate, ethyl acetate and water and obtained *o*-azoxyacetanilide. Elbs and Wogrinz (136) reduced *m*-nitroacetophenone in sodium acetate and alcohol, obtaining *m*-azoxyacetophenone and *m*-azoacetophenone.

When *m*-nitrobenzophenone was used an almost quantitative yield of azoxy compound was obtained. In no case was the ketone group reduced.

Brand and Steiner (137) studied the reduction of nitro compounds with hydrogen using palladium charcoal as the catalyst. Using 2 grams of nitrobenzene, 40 cc. of alcohol and 2 cc. of $2N$ sodium hydroxide they obtained only aniline; with 5 to 10 cc. of $2N$ sodium hydroxide, azoxybenzene and hydrazobenzene became the chief products. They obtained other azoxy compounds as well by this method. Using platinized calcium carbonate as the catalyst and hydrazine as the reducing agent, Busch and Schulz (138) reduced various nitro compounds in an alkaline solution. They found that the nature of the solvent exerted a notable influence on the course of the reaction. Increase in the quantity of the catalyst favored the formation of hydrazo and amino compounds. In methyl alcohol they obtained almost quantitative yields of azoxybenzene from nitrobenzene.

Though a neutral solution is, in general, less favorable than an alkaline or acid medium for the preparation of azoxy compounds, some cases are known in which they have been prepared in such a medium. Shoesmith and Taylor (139) using zinc and alcohol reduced *o*-nitrobenzyl alcohol to the hydroxylamine derivative and allowed this to oxidize in the air to *o*-azoxybenzyl alcohol. The *meta* and *para* compounds were prepared in the same way. The method is, of course, an indirect one. Bornstein (140) heated *p*-nitrosodimethylaniline with benzenesulfonyl chloride in benzene and obtained *p*-tetramethyldiaminoazoxybenzene. Nesbit (141) has shown that benzoin and some related compounds, in hot alcoholic solution under the influence of a trace of sodium ethoxide, reduce nitro compounds to azoxy compounds. The method is particularly satisfactory in the case of· *para* compounds. With *ortho* or *meta* substituted compounds the yields are smaller. The difficulty with the process is the separation of the azoxy product from the diketone formed from the reducing agent. Azoxy compounds were formed from *p*-nitrobenzylideneaniline, *p*-nitrobenzonitrile, *p*-nitrocinnamic ester, *p*-nitrostilbene and others.

XV. REDUCTION OF AZOXY COMPOUNDS

We have shown, in the discussion on the preparation of azoxy compounds, that an alkaline medium is most favorable for good yields. In acid solution the tendency is to favor the formation of amines. One would expect, therefore, to find that azoxy compounds have been most commonly treated with acid reducing agents. This is, in fact, true. Most of the work done has been with the object of determining the constitution of the azoxy compounds studied, through the isolation of the amines formed in acid solution. We have already given some illustrations of this method. Numerous illustrations could be given but a few will suffice. With tin and hydrochloric acid azoxybenzene is reduced to aniline, *p*-dichloroazoxybenzene with stannous chloride is reduced to *p*-dichlorobenzidine and *p*-aminoazoxybenzene with tin and hydrochloric acid gives aniline and *p*-phenylene diamine. Trimethylazoxyaniline with tin and hydrochloric acid gives *p*-aminodimethylaniline and methyl-*p*-phenylene diamine. Bromoazoxytoluene with tin and hydrochloric acid gives *p*-toluidine and *m*-bromo-*p*-toluidine.

Other reducing agents have been used also. Gabriel (142) treated *m*-dibromoazoxybenzene with alcoholic ammonium sulfide and obtained *m*-dibromohydrazobenzene. Schmidt (143) from *p*-nitroazoxybenzene by means of the same reagent obtained *p*-aminoazoxybenzene and *p*-aminoazobenzene, the former in larger quantity. Brunnemann (144) reduced *m*-azoxydisulfonic acid with ammonium sulfide to the corresponding azo compound. Sodium amalgam gave the same product in aqueous solution, but sulfur dioxide in aqueous solution had no action.

Elbs (145) reduced azoxybenzene electrolytically in acid solution to *p*-aminophenol. In the same way Lob (146) reduced azoxybenzene in alcohol and concentrated sulfuric acid to benzidine. Mercury was used as a cathode. Elbs and Schwartz (147) by electrolysis reduced *p*-diamino-*o*-azoxytoluene in dilute alcoholic solution with sodium acetate to *p*-diamino-*o*-hydrazotoluene in good yield.

Meldola and Andrews (148) reduced *m*-diaminoazoxybenzene

in alcohol with zinc dust and sodium hydroxide to the corresponding hydrazo compound. On exposure to the air the hydrazo body was oxidized to the corresponding azo body, a very general reaction. With potassium hydroxide the reduction did not stop at the hydrazo stage. Instead, *m*-phenylene diamine was the chief product.

Bigiavi (149) studied the action of nitroxyl on azoxy compounds. He found that azo compounds were formed. Azoxybenzene gave azobenzene, *p*-dinitroazoxybenzene gave *p*-dinitroazobenzene and *p*-mononitroazoxybenzene gave *p*-mononitroazobenzene.

Flürscheim (150) reduced *m*-dinitroazoxybenzene in a boiling absolute alcohol solution with ammonium sulfide: *m*-Nitroaniline, *m*-dinitroazobenzene and *m*-dinitrohydrazobenzene were the products.

XVI. THE ISOMERISM OF THE DIAZOTATES

One of the most interesting phases of the theory held by Angeli concerning the structure of azoxy compounds is the view now held by him that this theory applies also to the diazotates, whose structure for many years has been believed best explained by the *syn* and *anti* isomerism suggested by Hantzsch. The views of Angeli, expressed in a number of published researches of recent years, have been vigorously combated by Hantzsch and most particularly in a recent paper (102). In view of the contentious nature of the subject we have thought it unwise to introduce it here.

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THE HISTORY OF THE DISCOVERY OF THE AMINO ACIDS*

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INTRODUCTION

The observation that proteins, when they are subjected to the hydrolytic action of boiling acid, are decomposed into relatively simple crystalline substances, was made more than a century ago. At first only a few of these were distinguished but as time went on more of them were isolated, until today no less than twenty-one different, but allied, substances have been positively identified as products of the hydrolysis of proteins. No one protein is known to yield all of these but most proteins do yield some seventeen or eighteen. These substances are all, with two exceptions, α-amino acids, that is, they are substances of the general formula R—CHNH$_2$—COOH; the exceptions are proline and oxyproline, which are cyclic compounds and possess an α-imino group. Many of the properties of an individual amino acid are therefore common to all, and it is this close similarity in behavior that has rendered the problem of isolation so difficult. The radicals of the several amino acids are, however, widely different from each other. Three amino acids possess strongly acidic radicals and therefore titrate like ordinary carboxylic acids; three others have basic radicals and, of these, two are strong bases of the amine type; the rest are essentially neutral or, rather, amphoteric substances, but the relative strength of their acidic and basic groups is modified by the nature of the radical so that, even in this group, there is a considerable diversity in acid and basic properties.[1]

The capacity of the amino acids to form salts with each other, and their tendency to separate from solution in the form of mixed crystals, have prevented a clear understanding of the exact composition of the mixture of products derived from any one protein. The difficulties of the quantitative analysis of these mixtures can hardly be overemphasized, but, gradually, as knowledge of the qualitative composition increased, more and more progress has been made in the solution of the fundamental problem of the amino acid composition of proteins.

[1] A survey of the data on the dissociation constants of the amino acids is given by Kirk and Schmidt: Univ. Calif. Pub. Physiol. **7**, 57–69 (1929).

This paper is written with the object of showing in some detail how the advances in knowledge of the qualitative composition of proteins, with respect to the amino acids that they yield on hydrolysis, have come about. The term amino acids as used here is, of course, restricted to the substances of this class that have been found to result from the hydrolysis of proteins. The discovery of some of these has been the result of mere chance; others have been the fruit of a well-conceived hypothesis; many were found by investigators bold enough to depart from long-established custom and apply a new method or a new reagent. It will become clear, however, that the great rewards have come only to those who possessed intelligence, skill, and patience of the highest order; the discoverers of the amino acids are among the élite of science.

Proteins[2] have provided problems for investigation for centuries. The manufacture of cheese and the preparation of glue, the discovery that ammonia could be obtained by the distillation of horn, or of dung,[3] the use of egg-white or blood for the clarification of hot solutions, are all technical applications of protein chemistry and are the result of shrewd observations made, perhaps, many thousands of years ago. Modern protein chemistry dates, however, from 1820, when Braconnot prepared glycine from gelatin in the course of his attempt to see if proteins behave like starch and are decomposed by acids with the production of sugar. Progress at first was slow, but in recent years it has been rapid indeed; nevertheless the complete solution of the problem of protein composition has not yet been attained. Many improvements in the methods of amino acid analysis must be made, new amino acids must be sought for and isolated, and the theory of protein constitution must be brought to a far more highly developed state than it is at present, before we shall have much reason to be satisfied with our knowledge of these baffling substances.

[2] For a full discussion of the origin of the term protein see Vickery and Osborne: Physiol. Rev. **8**, 393–446 (1928).

[3] The name ammonia is derived from the deity Jupiter Ammon, near whose temple in Libya this substance was first prepared by the distillation of camel dung.

The amino acids that have been isolated from hydrolysates of proteins and so thoroughly investigated and described that no reasonable doubt of their presence in these hydrolysates can be entertained are shown in table 1. In forming this list two fundamental criteria have been adopted. In order that an amino acid shall be accepted as a definite product of the hydrolysis of proteins it must also have been isolated by some worker other than its discoverer and, further, its constitution must have been established by synthesis and by demonstration of identity between the synthetic product and the racemized natural product, or by actual resolution of the synthetic product and preparation of the optically active natural isomer. Although these criteria may appear somewhat arbitrary they are essential, unless one is prepared to accept a host of preparations that have been described, from time to time, as definite homogeneous products of the complete hydrolysis of proteins but for the exact nature of which convincing proof has not been presented. Too many errors have been committed to warrant any but a thoroughly conservative attitude towards newly discovered amino acids. Even the greatest leaders have made mistakes; Fischer himself described diaminotrioxydodecanic acid in 1904, but withdrew it in 1917.

Other criteria for acceptance are more or less obvious. The substance must be liberated by hydrolysis from a preparation of a protein of demonstrated purity and must be adequately characterized by analysis of salts and of typical derivatives. It is desirable, though not essential, that a synthetic peptide containing it should have been prepared and shown to be attacked by enzymes, and it should also have been demonstrated that the substance is oxidized in the animal body.

The amino acids are arranged on the left side of table 1 in the order of their discovery, either in nature or as the result of synthesis; the discoverer and date are also given. On the right side of the table the amino acids are arranged in the order of their discovery as products of the hydrolysis of proteins. In one or two cases there is some doubt as to who should have the credit for this demonstration. This is partly due to the difficulty of being sure of the identity of the preparations described in some

TABLE 1

Amino acids that have been demonstrated to be products of the hydrolysis of proteins

	AMINO ACID	EARLIEST OBSERVATION OF THE AMINO ACID		AMINO ACID	EARLIEST OBSERVATION OF THE AMINO ACID AS A PRODUCT OF HYDROLYSIS OF PROTEINS	
1	Cystine	Wollaston	1810	Glycine	Braconnot	1820
2	Leucine	Proust	1819	Leucine	Braconnot	1820
3	Glycine	Braconnot	1820	Tyrosine	Bopp	1849
4	Aspartic acid	Plisson	1827	Serine	Cramer	1865
5	Tyrosine	Liebig	1846	Glutamic acid	Ritthausen	1866
6	Alanine	Strecker (synthesis)	1850	Aspartic acid	Ritthausen	1868
7	Valine	von Gorup-Besanez	1856	Phenylalanine	Schulze and Barbieri	1881
8	Serine	Cramer	1865	Alanine	Weyl	1888
					(Schützenberger)	1879?
9	Glutamic acid	Ritthausen	1866	Lysine	Drechsel	1889
10	Phenylalanine	Schulze	1879	Arginine	Hedin	1895
11	Arginine	Schulze	1886	Iodogorgoic acid	Drechsel	1896
12	Lysine	Drechsel	1889	Histidine	Kossel} Hedin}	1896
13	Iodogorgoic acid	Drechsel	1896	Cystine	Mörner	1899
14	Histidine	Kossel} Hedin}	1896	Valine	Fischer	1901
15	Proline	Willstätter (synthesis)	1900	Proline	Fischer	1901
16	Tryptophane	Hopkins and Cole	1901	Tryptophane	Hopkins and Cole	1901
17	Oxyproline	Fischer	1902	Oxyproline	Fischer	1902
18	Isoleucine	Ehrlich	1903	Isoleucine	Ehrlich	1903
19	Thyroxine	Kendall	1915	Thyroxine	Kendall	1915
20	Oxyglutamic acid	Dakin	1918	Oxyglutamic acid	Dakin	1918
21	Methionine	Mueller	1922	Methionine	Mueller	1922

of the early papers or of the purity of the preparation of protein employed, and also because in at least one case, that of histidine, simultaneous independent and equally meritorious discoveries were made. These points are discussed in detail in the following pages.

A word as to the methods that were used by the various investigators should perhaps be added. Leucine, glycine, alanine, tyrosine, phenylalanine, glutamic acid, and serine were more or less chance products of fractional crystallization. Aspartic acid was discovered by the application of a new experimental method, the precipitation of its barium or calcium salt by alcohol; this method was likewise instrumental in the discovery of oxyglutamic acid. The discovery of the basic amino acids was also the result of the introduction of a new precipitant, phosphotungstic acid, and of the skillful use of silver nitrate. Proline, oxyproline, and valine, Fischer's contributions to the list, were the products of another new method and of a new point of view. Isoleucine was found because Ehrlich believed that a difference of a few degrees in specific rotation between his preparations of leucine and those of others must have an explanation. Tryptophane was first isolated by methodically following a color reaction during a fractionation with a new reagent. Thyroxine and iodogorgoic acid were detected by analyses for iodine. Cystine was found in proteins by a man who was convinced that it must be there. Methionine was isolated as a result of an investigation of the nature of a substance in protein hydrolysates that stimulated the growth of certain bacteria. This search was side-tracked by the observation that sulfur was present, in the active fraction, in an unfamiliar type of combination.

CYSTINE

$$S—CH_2—CH(NH_2)—COOH$$
$$|$$
$$S—CH_2—CH(NH_2)—COOH$$

Although cystine was shown as recently as 1899 to be a product of the hydrolysis of proteins, it was the first amino acid to be discovered. In 1810 Wollaston (43) described a substance that

he had found in a new type of urinary calculus. It was soluble in both acids and alkalies and separated from alkaline solution in hexagonal plates on acidification with acetic acid. It burned with a characteristic unpleasant smell and a bluish flame; ammonia and carbon dioxide were present among the products of dry distillation, but he did not note the presence of sulfur. Wollaston wrote, "From the ready disposition of this substance to unite with both acids and alkalies, it would appear to be an oxide; and that it does, in fact, contain oxygen is proved by the formation of carbonic acid on distillation. . . . . I am therefore inclined to consider it as an oxide, and since both the calculi that have yet been observed have been taken from the bladder, it may be convenient to give it the name of *cystic oxide*, which will serve to distinguish it from other calculi."

Occasional references[4] to the occurrence of similar calculi are found in the early literature, Walchner's (42) identification of the substance in a calculus from a boy's urine being perhaps the most clearly substantiated. Lassaigne (25) claimed to have identified it in a calculus secured from a dog but his description is far from convincing. He described white transparent leaves secured by the evaporation of the solution in ammonia, but did not mention that the leaves were six-sided. The shape of cystine crystals is so striking that he could hardly have failed to refer to it if his substance had been in fact cystine. Furthermore the analytical figures he gave bear no relation whatever to the composition of cystine, and Berzelius (5) dismissed his identification as improbable.

The name cystine appears in Berzelius' Jahresbericht for 1832 in connection with a report on an observation by Venables of a urinary calculus (6). The exact date on which the new name was first used is not certain but, in the French edition of his textbook published in 1833 (7), Berzelius described the substance discovered by Wollaston and pointed out that, although cystic oxide resembled certain metallic oxides in regard to its solubility, the term oxide was inappropriately used as a designation for an

[4] An extensive bibliography of early observations of cystine stones is given by Liebig, Poggendorff and Wöhler (26), and also by Gmelin (20).

organic substance since most of these contain oxygen; "je me suis donc permis de changer le nom qu'avait proposé cet homme distingué." Berzelius is therefore responsible for the name cystine, and it is probable that he first used it when preparing the manuscript of the 1833 edition of his treatise.

The earliest recorded analysis of cystine was carried out by the distinguished English scientist William Prout (1785–1850), the discoverer of hydrochloric acid in gastric juice (37), and the man whose speculation that the atomic weights of the elements (35) are integral multiples of that of hydrogen has been so strikingly revived in recent years. Prout (36) analyzed a purified

TABLE 2

Analysis of cystine

		THEORY, ATOMIC PROPORTIONS	PERCENTAGE COMPOSITION	
			Found	Theoretical (modern atomic weights)
			per cent	*per cent*
Hydrogen............	3 atoms	3.75	5.00	5.03
Carbon.............	3 "	22.50	30.00	29.96
Nitrogen............	1/2 "	8.75	11.66	11.66
Oxygen.............	4 "	40.00	53.33	26.65
				26 70 (Sulfur)
		75.00	100.00	100.00

specimen of cystine secured from calculi collected by Marcet. His results were so extraordinary that they merit quotation in full and are given in table 2. Sulfur was not detected but its presence appeared to have had no effect upon the accuracy of his analysis!

Baudrimont and Malaguti (1) in 1837 announced that cystine contained sulfur. At Baudrimont's request Pelouze carried out analyses of a specimen of the same material for carbon, hydrogen, and nitrogen, and obtained results that confirmed Prout's. Pelouze informed Liebig of Baudrimont's discovery and also sent him a small sample of purified cystine. Liebig turned this material, together with a cystine calculus already in his possession, over to Thaulow for analysis. Thaulow conducted his final

analyses for carbon, hydrogen, nitrogen, and sulfur directly on the calculus without purification, and used Pelouze's purified material for practice on the method for nitrogen! His results (40), nevertheless, were in remarkably close agreement with the older analysis of Prout[5] and with the recent one of Pelouze, and the formula $C_6H_{12}N_2O_6S_2$[6] was suggested as being in closest agreement with the analytical results. Determinations of the nitrogen and the sulfur content of purified cystine were also made by Marchand in 1839. He found 11.88 per cent nitrogen and 25.55 per cent sulfur.

It is difficult to follow the changes in the formulation of organic compounds through the period when the atomic weight of carbon to one worker was 6, to another 12, when oxygen was taken sometimes as 8, sometimes as 16, and when there was no clear appreciation of the concept of molecular weight. Thaulow's correct formula was later written $C_6H_6NS_2O_4$ (O = 8, C = 6, S = 16) and, because the sum of the hydrogen and nitrogen atoms was odd, Gmelin (20), in his celebrated Handbuch, in 1852, arbitrarily changed this formula to $C_6H_7NS_2O_4$. Gerhardt (19) was more cautious and gave $C_{12}H_{12}N_2O_8S_4$ in his Lehrbuch published the following year; this is simply Thaulow's formula with the above mentioned atomic weights. Gerhardt affixed a question mark to this formulation, however. Grote (21) in 1864 carried out analyses for carbon, hydrogen, and sulfur on purified cystine and obtained figures in very close agreement with Gmelin's altered formula. He said, "Es ist hiernach unzweifelhaft dass bei der Analyse von Thaulow ein Verlust von Wasserstoff stattfand."

Dewar and Gamgee in 1871 (9) made the first attempt to

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[5] In the French translation of Thaulow's paper Prout's name is misspelled Proust and this misspelling is repeated in Baudrimont and Malaguti's paper which follows Thaulow's. Evidently some confusion with the name of the former distinguished professor at Madrid was present in the mind of the proof reader.

[6] Thaulow employed the following atomic weights: O = 100, C = 76.4, N = 88.5, H = 6, S = 201. These are equivalent to O = 16.0, C = 12.2, N = 14.16, H = 0.91, S = 32.06. Thaulow's symbols therefore had the same meaning to him that they do to us today; his formula is correct. Incidentally it may be interesting to recall that the use of unity for the atomic weight of oxygen was first suggested by Wollaston; the value 100 was used by Berzelius later.

ascribe a structural formula to cystine. They treated cystine with nitrous acid and isolated a silver compound which they thought to be silver pyruvate. They observed the formation of ammonia and free sulfur when cystine was heated to 150° with alkali, and the formation of hydrogen sulfide under the action of nascent hydrogen. The formula CH_2NH_2—CS—COOH appeared to express these reactions best, although they carried out no analyses to verify this composition.

Baumann and Preusse in 1881 (4) observed the elimination of bromophenylmercapturic acid by dogs to which bromobenzene had been administered. Hydrolysis of this yielded a substance that they regarded as bromophenylcystine, inasmuch as the formula they obtained by analysis could be accounted for by substituting C_6H_4Br for one of the hydrogen atoms of cystine, $C_3H_7SNO_2$. A study of the behavior of this substance, especially its decomposition to bromophenyl mercaptan, ammonia, and pyruvic acid under the action of alkalies led them to propose for it the formula,

$$S—C_6H_4Br$$
$$|$$
$$CH_2—C—NH_2$$
$$|$$
$$COOH$$

and, as a logical deduction, the formula,

$$SH$$
$$|$$
$$CH_2—S—NH_2$$
$$|$$
$$COOH$$

was proposed for cystine. In support of this formula they quoted three unpublished analyses by Hoppe-Seyler. One of these analyses gave a figure for hydrogen that agreed exactly with the requirements of theory, the other two were low. They pointed out that, if Dewar and Gamgee's formula were correct, methylamine should be a product of the decomposition of cystine; this, however, was not the case.

E. Külz was the next to take up the problem of the composition

of cystine. In 1884 (23) he reviewed all the previous analyses and pointed out that hydrogen determinations usually give somewhat high values. Two of Hoppe-Seyler's results, on the other hand, were low if the correct formula of cystine is $C_3H_7NSO_2$. Külz had available no less than 26 grams of cystic calculi as well as supplies of urine from a cystinuric patient. Purified preparations of cystine from this material were subjected to analysis. He wrote, "Für die Formel $C_3H_7NSO_2$ ist der Wasserstoff in sämmtlichen Analysen zu niedrig ausgefallen . . . . so stimmen sämmtliche Analysen gut, am besten speciell der Wasserstoff, ... zur Formel $C_3H_6NSO_2$, eine Formel, die freilich dem oben erwähnten Gesetze widersprechen würde. Ob sie die richtige ist, oder ob sie gar verdoppelt werden muss, wird erst die Synthese des Cystins endgültig entscheiden können."

It is indeed remarkable that the presence of cystine among the products of hydrolysis of proteins should have escaped notice for so many years. The presence of sulfur in proteins was recognized by Scheele and by Fourcroy in the eighteenth century, and attention was focused on this fact by the speculations of Mulder (41). Cramer, in 1865, suggested that there was an analogy between serine, his newly discovered amino acid from silk, and the cystine of urinary calculi, and it is clear that E. Külz, in 1886, suspected that cystine was present among the decomposition products of proteins.

A number of reasons may be advanced to account for the failure to find cystine in proteins. Up to 1873 sulfuric acid had been almost universally employed for the hydrolysis of proteins. The acid was usually removed as calcium sulfate and the cystine formed by the hydrolysis would, almost inevitably, be lost in the precipitate. Moreover, few hydrolyses of proteins of high cystine content, such as hair or horn, seem to have been attempted in the early days. The introduction of hydrochloric acid as a hydrolyzing agent by Hlasiwetz and Habermann (22) in 1873 would probably soon have brought cystine to light if stannous chloride had not almost invariably been added to the mixture in order to keep down humin formation during the hydrolysis. Cystine was thereby reduced to the much more soluble cysteine and thus es-

caped observation. Even Mörner, who hydrolyzed horn with hydrochloric acid and did not add tin, failed at first to hit upon the simple expedient of neutralizing the hydrolysate with sodium carbonate.

E. Külz of Marburg, in 1886, requested Richard Külz, his nephew and assistant, to investigate the problem "ob bei der Einwirkung des pankreatischen Saftes auf Eiweisskörper ausser den bis jetzt bekannten noch andere Spaltungsproducte entstehen, und besonders mit der Lösung der Frage, in welcher Form der Schwefel dabei auftritt." Richard Külz unfortunately died[7] before the work was completed and his observations remained unpublished until 1890, when E. Külz published a short paper (24) in which he stated that R. Külz "ist . . . . auf einen Befund gestossen, der mir schon jetzt der Mitteilung werth scheint." Külz had prepared a mixture of 290 grams of crude fibrin and 270 grams of minced pancreas which was protected from decomposition with salicylic acid and allowed to digest for about 48 hours, part of the time at room temperature. No hydrogen sulfide was evolved. The mixture was filtered and evaporated to half its volume, filtered, and allowed to stand. A white deposit separated that was insoluble in water, but when this was dissolved in ammonia and the solution was evaporated, a crust of well-formed, six-sided plates separated which were insoluble in acetic acid. A second crystallization from ammonia gave six-sided tablets. The material burned with a greenish-blue flame, contained both sulfur and nitrogen and was strongly levorotatory. E. Külz unhesitatingly identified it as cystine, a judgment for which his previous experience had thoroughly qualified him. He pointed out, however, that the experiment did not decide whether the cystine had been originally present in the pancreas employed, whether it arose from the action of the pancreas on the fibrin, or whether it had arisen from bacterial action. "Ja, die Möglichkeit, dass das Cystin dem angewandten Fibrin angehaftet habe, kann ohne Weiteres nicht absolut ausgeschlossen werden." Thus the first attempt to prepare cystine from protein ended in uncertainty.

[7] Richard Külz disappeared during a solitary holiday expedition to the Beerberg in the Thüringer Wald; no trace of him was ever discovered in spite of the most careful search (personal communication from Prof. Fritz Külz, Kiel).

It is interesting to trace the evil fortune that pursued the investigators of cystine. Early workers lost any that may have separated from their hydrolysates in the precipitate of calcium sulfate which they filtered off and discarded. Later investigators inadvertently reduced the cystine to cysteine and so missed it. R. Külz died before he could complete an investigation that promised success. Emmerling (12), a bacteriologist, in 1894, observed cystine admixed with the tyrosine which he had prepared by the hydrolysis of horn, but he failed to follow up his observation with a positive identification. Suter (39) in 1895 discussed the whole problem of the presence of cystine in proteins in the light of Baumann's experiments on mercapturic acid elimination and of Cloetta's, Scherer's and Drechsel's observation of cystine in animal and human livers (8, 38, 10). Cystine was almost certainly an intermediary product of protein metabolism but "die Frage, ob das Cystin sich bei directer hydrolytischer Spaltung . . . . aus dem Eiweiss abspaltet, ist noch nicht gelöst." Baumann had suggested this problem to Suter but, by the grimmest mischance, had given him for investigation, not horn, nor hydrolysates of horn, but filtrates that remained over after work carried out years before on the isolation of tyrosine from horn! Very little cystine could have remained in them; most of the sirupy solutions he provided were acid, but a few had become alkaline and molds had formed on these.

The selection of horn was made because of Suter's own observation that this material contained unusually high proportions of sulfur in a form that yielded sulfide when the material was heated with alkaline lead acetate. Inasmuch as cystine behaved in an analogous fashion it seemed reasonable to suppose that horn should yield this amino acid in considerable amounts. Suter's attempts to isolate cystine were well conceived. He employed mercuric chloride as a reagent and found that a substance that contained both sulfur and nitrogen was precipitated. The substance likewise gave the sulfide reaction with alkaline lead acetate and, furthermore, gave an evanescent blue color with ferric chloride and a violet color with copper sulfate. He satisfied himself that cystine, after reduction to cysteine, gave somewhat

similar color reactions but failed in his attempts to isolate from the horn hydrolysates the material that was responsible for these reactions. He pointed out that both reactions are given by α-thiolactic acid and, in fact, isolated this substance from one of the tyrosine mother liquors that had molded. He concluded, somewhat ruefully, "vielleicht decken spätere Untersuchungen die Bedingungen auf, die erfüllt sein müssen, damit Cystin unter den Spaltungsproducten von Eiweisssubstanz auftritt."

Baumann added a few remarks to Suter's paper (3) in which he drew attention to the increasing number of sulfur compounds that had been observed to result either from metabolism, or from the decomposition of proteins, and to the interest in the precursor of these which must be present in the protein molecule. "Man könnte daran denken, dass es sich um eine geschwefelte Asparaginsäure handelt:

$$\begin{array}{c} CH_2\text{---}COOH \\ | \\ HS\text{---}C\text{---}COOH \\ | \\ NH_2 \end{array}$$

welche sehr wohl die Stammsubstanz des Cystins, des Cysteins, der Mercaptursäure, der Thiomilchsäure, und des Aethylsulfids sein könnte." This sums up the results of eighty-five years of study of cystine. Baumann was entirely correct only in his conviction that there was a mother substance in proteins from which all these products were derived.

But the ill-luck that had pursued the investigators of cystine could not, according to the law of probability, always reign supreme, although, as will presently appear, it did not immediately relax its influence. K. A. H. Mörner, in 1899 (March 8), reported to the Swedish Academy (28) that he had obtained cystine by the acid hydrolysis of horn. This announcement was followed by a paper in the Zeitschrift für physiologische Chemie (29) in which full details were given. Carefully purified horn shavings were heated with approximately 15 per cent hydrochloric acid at a temperature of 90–95° for one, or for two, weeks. The selection of these conditions of hydrolysis was made so as to avoid, as

much as possible, the decomposition of the sulfur compounds and the production of hydrogen sulfide. After filtration and decolorization the acid was distilled off, the residue was dissolved in water, and neutralized with lead oxide. Alcohol was then added to complete the precipitation of the lead compounds. The precipitate was decomposed with oxalic acid and the acid solution was neutralized with ammonia, or else with calcium carbonate; it was then treated with an excess of ammonia, and filtered. The ammoniacal solution was evaporated at low pressure, whereupon cystine and tyrosine crystallized. These were separated by appropriate treatment with dilute ammonia. The presence of cystine in the different fractions was followed by application of the lead-blackening test and by the behavior towards Millon's reagent, which likewise gave information of the presence of tyrosine. The cystine was finally recrystallized from dilute ammonia by evaporation over sulfuric acid; Mörner says, "die Menge des Cystins war nicht gering. . . . . Insgesammt erhielt ich . . . . beinahe 2 1/2% der trockenen Hornsubstanz." The product was thoroughly identified by analysis and reactions.

This paper contains a vast amount of accurate information on the chemical behavior of cystine. Mörner observed the formation, when the hydrolysis of the protein was prolonged, of a more soluble and less strongly levorotatory variety of cystine which crystallized in needles; he found that residues of cystine in the mother liquors could be recovered by precipitation with copper acetate and by mercury salts and that these metal compounds, on decomposition with hydrogen sulfide, yielded much of their cystine in the form of cysteine. He discussed the meaning of the changes in optical activity of the cystine with their associated changes in solubility, but pointed out that the chemical properties of the two types of cystine were identical; "Alles spricht für die Annahme einer Stereoisomerie." He concluded, "Aus den oben mitgetheilten Untersuchungen geht hervor dass man durch hydrolytische Spaltung der Hornsubstanz bei der Einwirkung von Salzsäure Cystin in beträchtlicher Menge darstellen kann . . . . in der Hornsubstanz gewissermassen eine Cystingruppe präformirt vorfindet, oder jedenfalls eine Atomgruppe, welche leicht in Cystin übergeht."

Shortly after the appearance of his first paper Mörner improved the method of preparing cystine from proteins. In a lecture given before the International Congress at Paris in 1900 he described the substitution of sodium hydroxide or ammonia for lead oxide in the neutralization of the hydrolysate and the great improvement in yield that resulted. He obtained 6.8 per cent of cystine from horn, 6.0 per cent from egg membrane, and 12.6 per cent from human hair. He had also obtained a 1 per cent yield from serum albumin. These were results that are difficult to match even today. In his audience was Gustav Embden, then a young assistant at Zürich. During the previous year Embden, without knowledge of Mörner's work, had hydrolyzed horn with hydrochloric acid and had isolated cystine! When this was brought to his attention Mörner at once recognized Embden's originality and generously permitted him to publish the essential points of the lecture as an introduction to the paper Embden was then preparing (11). Embden had boiled horn for 5 to 6 hours with concentrated hydrochloric acid; the hydrolysate was then neutralized in the cold with sodium hydroxide and, after standing for 24 hours, was filtered from the dark brown precipitate or "Melaninniederschlag." Embden unfortunately did not investigate this material; it must have contained much of the cystine. The filtrate was decolorized and evaporated and a number of successive crops of crystals were removed. By taking advantage of the greater solubility of the impurities in very dilute nitric acid Embden succeeded in isolating cystine from these fractions. He also isolated cystine from egg albumin and serum albumin, and obtained reactions for cystine in edestin. Cystine was thereby established as being a generally distributed amino acid rather than a product only of the insoluble keratins.

The investigations of Baumann and Preusse already mentioned had led to the conclusion that the sulfur and nitrogen atoms of cystine were both attached to the same carbon atom. In 1884 Baumann (2) obtained chemical evidence that Thaulow's original formulation of cystine ($C_6H_{12}N_2S_2O_4$) and Külz' idea of a double molecule were correct. When cystine was treated with tin and hydrochloric acid, it was converted into an easily oxidized sub-

stance of approximately the same ultimate composition but of entirely different properties.   The relationships were obviously analogous to those between a mercaptan and a disulfide and the two substances were therefore formulated as follows:

$$
\begin{array}{ccc}
& \text{NH}_2 & \\
& | & \\
\text{CH}_3\text{---C---SH} & & \\
& | & \\
& \text{COOH} &
\end{array}
\qquad
\begin{array}{ccccc}
\text{NH}_2 & & & \text{NH}_2 & \\
| & & & | & \\
\text{CH}_3\text{---C---S---S---C---CH}_3 & & & & \\
| & & & | & \\
\text{COOH} & & & \text{COOH} &
\end{array}
$$

Cysteine   Cystine

Baumann wrote, "Um die Beziehungen dieser Substanz zu dem Cystin zu bezeichnen, nenne ich diese Reduktionsprodukt des Cystins: Cystein."   The mercapturic acids were obviously substitution products of cysteine rather than of cystine and their nomenclature was therefore corrected.

The formulation of cysteine as α-thio-α-aminopropionic acid did not go unchallenged.   Neuberg (33), in 1902, pointed out that the production of pyruvic acid from cystine was no criterion for the position of the thiol group, as numerous cases were known in which a keto group resulted from conversions of hydroxyl compounds, probably with the intermediate formation of an ethylene oxide ring.   An analogous reaction might occur in the case of cystine.

Neuberg wished to convert the labile thiol group into the more stable sulfonic acid group; to this end he subjected cysteine, prepared from cystine calculi, to the action of nitric acid.   The product isolated turned out to be isethionic acid, $SO_3H\text{---}CH_2\text{---}CH_2\text{---}OH$, which could readily be accounted for by the action upon the amino group of the nitrous acid produced during the oxidation of the thiol group.   It was evident that the thiol and the amino groups of cysteine, and therefore of cystine, were on different carbon atoms and only two possibilities for the formula of cysteine could be admitted.   It might be α-thio-β-aminopropionic acid or α-amino-β-thiopropionic acid.   Of these the second was more probable in view of the obvious relationship to serine.   Neuberg suggested that, although this was true for cystine derived from calculi, the identity of this substance with cystine derived from proteins was not yet demonstrated.

While Neuberg's paper was still in proof an announcement was made by Friedmann (16) that he had converted protein cystine into taurine, SO_3H—CH_2—CH_2—NH_2, a demonstration that cystine from another source contained thiol and amino groups on different carbon atoms. The full description of this work came the following year (17). Friedmann likewise pointed out the weakness of Baumann's argument, and then described experimental work that thoroughly demonstrated cystine to be the disulfide α-amino-β-thiopropionic acid. Cystine, when treated in concentrated hydrochloric acid solution with sodium nitrite, was converted to dichlorodithiopropionic acid; reduction with zinc and hydrochloric acid removed the chlorine and gave a thiolactic acid. This was either α- or β-thiolactic acid. An attempt to identify this as its benzyl compound failed, and Friedmann therefore oxidized it with ferric chloride to the dithio acid; this was identical with the product obtained from β-iodopropionic acid with potassium hydrogen sulfide. The sulfur of cystine was therefore in the β position; the nitrogen might be either α or β. Friedmann regarded the former as more likely. He oxidized cystine with bromine and prepared the new substance cysteic acid,

$$CH_2\text{—}SO_3H$$
$$|$$
$$CH\text{—}NH_2$$
$$|$$
$$COOH$$

thoroughly investigated it and its salts, proved that it was a sulfonic acid, and finally converted it[8] to taurine, NH_2—CH_2—CH_2—SO_3H, by heating it with water at 235°. The constitution of taurine was known; sulfur and nitrogen were on different carbon atoms. The sulfur in cystine was in the β position, hence the

[8] Gortner and Hoffman (Gortner, R. A. and Hoffman, W. F.: Sulfur in Proteins. J. Biol. Chem. **72**, 433–48 (1927)) have reported that they were unable to duplicate Friedmann's synthesis of taurine. Lewis and Lewis (Lewis, G. T. and Lewis, H. B.: The Metabolism of Sulfur. XI. Can Taurine Replace Cystine in the Diet of the Young White Rat? J. Biol. Chem. **69**, 589–97 (1926)) have also reported difficulty, but state that a successful preparation had been made by one of their associates. The matter is under investigation in the laboratory of one of the writers (8).

nitrogen must be in the α position and the formula was complete in every detail.

The accuracy of this formula was promptly confirmed by the synthesis of cystine by E. Erlenmeyer, Jr. (13, 14) from benzoyl serine. When treated with phosphorus pentasulfide a thiol group was introduced in place of the hydroxyl, and subsequent hydrolysis of the benzoyl group and oxidation gave inactive cystine.

During the early years of the present century a controversy arose as to the identity of the cystine from proteins with that from urinary calculi. Neuberg and Meyer (34) detailed a large number of points of difference between cystine from the two sources and Mörner's work (30, 31, 32), in which he obtained α-thiolactic acid from cystine, served to strengthen the view that stone cystine was the disulfide of α-thio-β-aminopropionic acid. Fischer and Suzuki (15), however, showed that Neuberg and Meyer's points of difference were all due to the presence of tyrosine in their preparation of stone cystine, and Gabriel's synthesis (18) of α-thio-β-aminopropionic acid, which differed in many ways from cysteine, finally settled the matter. Protein cystine and stone cystine are identical.

It required ninety-five years of investigation finally to settle the origin and constitution of cystine, but the problems presented by this fascinating amino acid are still far from solved. During the year 1930 a single journal, the Journal of Biological Chemistry, published no less than seventeen papers dealing directly with the chemistry or metabolism of cystine, and this represents but a small part of the total volume of work, for the most part of the highest quality, that was published on this substance during that year. Probably no other amino acid has so extensive a bibliography nor has attracted so much talent and, it is only fair to add, probably no other is so difficult to understand.

LEUCINE

$$\begin{array}{c} CH_3 \\ \diagdown \\ \diagup \\ CH_3 \end{array} CH—CH_2—CH(NH_2)—COOH$$

In 1819 Proust (60) described a series of experiments, on different types of fermentation, that had been conducted in connection with a study of the principle to which different types of cheese owe their flavor. He had observed that gluten from wheat flour undergoes a spontaneous change which bears some analogies to that by which sweet substances become converted into new products and that, in addition to carbon dioxide, ammonia and acetic acid, two new substances were produced ". . . . premièrement, un acide particulier que j'ai cru pouvoir nommer *acide caséique*; puis un second produit que j'appelerai *oxide caséeux*." He suggested these names as convenient designations pending the outcome of further investigation which should decide whether or not the substances were indeed new.

Products of exactly the same nature were found among the products of fermentation of milk curds and he pointed out that, because of this, it would not be necessary to work with wheat gluten in order to obtain them. He commented at some length upon the good fortune that led him to study gluten before investigating milk curd, since otherwise he would never have observed the production of these substances by the fermentation of the former material. As he quaintly remarks, "Supposons, en effet, que j'eusse trouvé d'abord l'acide caséique dans le caillé, quel attrait d'utilité pouvait alors m'appeler vers la fermentation de la glutine? Aucun, quand j'y réfléchis, puisque la glutine fermentée n'est bonne à rien, puisque son fromage ne peut jamais entrer sur nos tables en concurrence avec celui de nos laitages."

"Oxide caséeux" was obtained from a water extract of fermented gluten. The extract was evaporated to a sirup and "sans retirer cette masse de la bassine, on la couvre d'alcool, on l'agite; elle se trouble, et il s'en sépare une poudre blanche abondante qu'on achève de laver sur le filtre avec de l'alcool, jusqu'à ce qu'on ne lui trouve plus de saveur fromageuse." By following a similar procedure, Proust was also able to isolate "oxide caséeux" from milk curds. The filtrate, after removal of his first crop of crystals, gave the product which he termed "l'acide caséique." As will appear from Braconnot's work, this was probably also leucine.

The chemical tests which Proust carried out were entirely of a qualitative nature.   He observed that the substance was organic; he noted its solubility in various solvents, and its precipitability by salts of the heavy metals.   It must have been evident to him that the substance contained nitrogen because on heating "acid caséique" with aqua regia "cet acide, chauffé dans une retorte, fournit les produits ordinaires aux matières animales, tels que carbonate d'ammoniaque, huile, hydrogène huileux et un charbon volumineux, sans aucune trace d'odeur prussique."

Although there is little doubt that the material isolated by Proust was essentially leucine, it is necessary to bear in mind that it was undoubtedly contaminated with other amino acids.   The "leucine" of Proust, Braconnot and nearly all the subsequent investigators to the end of the nineteenth century was a white powdery substance which, under the microscope, was seen to consist of tiny nodules or balls of needles.   Figures of this material may be found in many of the older textbooks and it is accurately described by Proust in the following terms: "D'abord, pour le purifier davantage, on le fait dissoudre dans l'eau bouillante, on filtre immédiatement, on évapore, et vers la fin on voit se former une nappe et des encroûtements qui s'accumulent sur les bords.   Après le refroidissement, on jette tout sur un filtre afin de retenir dans l'eau mère des restes de caséate ammoniacal; enfin, on lave avec un peu d'eau froide, et l'on met a sécher. Notre produit a la légèreté, la blancheur et le spongieux de l'agaric blanc des drogueries. . . . . Trois dissolutions consécutives, des évaporations rapides ou spontanées n'ont rien changé à ses apparences. . . . . Les fragments en sont si légers qu'ils surnagent l'eau froide et l'eau bouillante; et c'est vers le 60me degré qu'ils commencent à s'y dissoudre: l'eau ne semble pas les mouiller."   And further, "l'oxide sous la forme de concrétions blanches globuleuses se présente à la vue, dans les fromages de glutine qui n'ont pu se dessécher."

A year after Proust isolated "oxide caséeux" from cheese, Henri Braconnot (45) obtained leucine by acid hydrolysis of muscle fibre and of wool.   It was he who gave the name of leucine to the white crystalline substance which separated from the hydrolysate

on addition of alcohol.   The crude protein was treated with twice its weight of concentrated sulfuric acid and allowed to stand for 24 hours.   The mixture was then boiled for 5 hours, cooled, and saturated with calcium carbonate.   The filtrate from the calcium sulfate was evaporated to a sirup.   The final step in the isolation is best described in his own words: "On a fait bouillir, à plusieurs reprises, cet extrait avec de l'alcool à 34° Beaumé: les liqueurs réunies ont laissé déposer, par le refroidissement, environ un gramme d'une matière blanche particulière, que je désignerai provisoirement par le nom de leucine (λευκός blanc)."   He made no analysis of the material nor did he realize that there was a relation between it and Proust's "oxide caséeux."   In fact, no mention is made in Braconnot's paper of Proust's previous work.

In 1827, Braconnot (46) published a paper in which are given the results of a repetition of Proust's experiments on the fermentation of cheese.   His opinion of Proust's work and his reasons for reinvestigating this subject are given in his opening paragraph. "Personne ne contestera à Proust les immenses services qu'il a rendus à la science; mais on est forcé de convenir que ses derniers travaux n'offrent pas toujours la précision et l'exactitude qu'on devait attendre d'un aussi habile chimiste.   C'est après avoir fait quelques recherches sur l'hordeine, et m'être convaincu qu'elle n'est qu'un composé d'amidon, de matière animale et de fibre ligneuse, que je me suis déterminé a répéter les expériences du même chimiste sur la fermentation du caillé."   To 750 grams of milk curds which had been drained, but not washed, he added a liter of water and permitted the mixture to stand at room temperature for a month.   He then distilled part of the fluid to remove the putrid odor, filtered off a coagulum which had formed as the result of heating, and concentrated the fluid to a sirup. Then, "cette masse, délayée avec de l'alcool à 37°, a été partagée en deux matières, très-improprement appelées, la première *oxide caséeux*, et la seconde, retenue en dissolution dans l'alcool, *caséate d'ammoniaque*."   The "oxide caséeux" was purified by recrystallizing from water.   He noted its relative insolubility in water, that it left no residue on ignition, that when slowly heated an ammoniacal product was formed which turned red litmus paper

blue and effervesced when treated with acids, and that it was more soluble in hydrochloric acid than in water. He did not have facilities at his disposal for carrying out an analysis of the product and therefore was unable to determine its composition. It appeared to him that, inasmuch as "oxide caséeux" did not contain very much oxygen, the name was not appropriate and, "comme elle semble se former toutes les fois qu'on abandonne des substances animales à la putréfaction, je propose de la nommer *aposépédine* de ἀποσῆπεδων, resultant de la pourriture." Thus, without realizing any relationship, he gave another name to a product which seven years previously he had termed leucine.

Braconnot next examined the substance that remained in solution when alcohol was added to the sirupy hydrolysate and that had been named "caséate d'ammoniaque" by Proust. As the result of various experiments, Braconnot concluded that it was composed of "acide acétique libre; aposépédine; matière animale soluble dans l'eau et insoluble dans l'alcool rectifié (osmazome); matière animale soluble dans l'eau et dans l'alcool; huile jaune, fluide, très-âcre; résine brune, plus sapide; acétate de potasse; muriate de potasse; acétate d'ammoniaque, des traces."

The experiments of Braconnot are cited at some length because they illustrate the difficulties that confronted the early chemists who worked with proteins and amino acids and also show how slowly progress was made in this field.

In 1839, Mulder (59) found that the leucine Braconnot had obtained by acid hydrolysis of proteins could also be obtained by alkaline hydrolysis of similar substances. He noted its solubility and melting point and observed that, when heated at 108° with lead oxide, it lost no weight; it therefore contained no "chemisch gebundenes Wasser." Mulder alone of the early investigators appears to have isolated leucine in approximately pure form. He described one of his preparations as consisting of gleaming white plates that possessed a greasy feel and were difficult to moisten with water. He assigned to leucine the formula of $C_{12}H_{24}N_2O_4$ with a molecular weight of 1644.035[9] and, further, found that

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[9] See atomic weights employed by Thaulow as given in footnote 6. The formula was doubled because the sum of the hydrogen and nitrogen atoms must be an even number.

100 parts of leucine combine with 27.6 parts of hydrochloric acid: save for a slight error in the estimation of hydrogen, this formula for leucine is double that accepted today.

Mulder (59) was the first to realize that Braconnot's "aposépédine" and leucine were one and the same substance. The following quotation leaves no doubt as to his ideas; "Es besitzt folgende Eigenschaften, wie ich sie an dem aus Leim, Fleisch und Eiweiss entweder vermittelst Schwefelsäure oder Kali bereiteten oder endlich dem aus verfaultem Käse abgeschiedenen Leucin beobachtete."

In 1846, Liebig (57) showed that leucine and another substance, which later proved to be tyrosine, were formed when casein was fused with potassium hydroxide.

Iljenko (55), in 1847, repeated the work of Braconnot but used casein that had been purified. Estimation of nitrogen in his "aposépédine" gave values nearly the same as those of Mulder; he believed therefore "dass Mulder mit Recht das Aposepedin bloss als unreines Leucin betrachtet." That leucine could be obtained from casein, either by bacterial putrefaction or by the action of alkali, was clearly seen by Iljenko. ". . . . So liefert auch das Casein das Leucin und die flüchtigen Säuren bei seiner Fäulniss und bei Einwirkung des Alkalis in höher Temperatur." He believed that in the process of putrefaction "Leucin ist durch Oxydation entstanden. . . . . ."

Laurent and Gerhardt (56) analyzed leucine the following year. Their preparation was isolated from the putrefactive products of milk curds and the results of the analysis indicated the formula of leucine to be $C_6H_{13}NO_2$. This is the first correct formula of leucine to be published. They classified leucine in the series $C_nH_{2n+1}NO_2$; glycine constituted the second member of this series, sarcosine the third, and leucine the sixth. At this date only five of the amino acids had been isolated and the structure of none of these had been elucidated; consequently there is little wonder that the system of classification was arbitrary. However, the germ of a rational basis of classification was beginning to appear.

Liebig (57) had observed that valeric acid, together with am-

monia and hydrogen, were obtained by the fusion of leucine with caustic alkali; Laurent and Gerhardt demonstrated that, by a similar procedure, sarcosine yielded acetic acid and glycine yielded formic acid.   Strecker (64), on treating leucine with fuming nitric acid, obtained oxycaproic acid.   Shortly after the publication of Laurent and Gerhardt's paper, Cahours (47) took up the consideration of the relationship of leucine to "aposépédine" and showed from analyses that the two products were identical and could be represented by the formula $C_{12}H_{13}NO_4$ (equivalent to $C_6H_{13}NO_2$).   His classification of leucine and glycocoll was the same as that previously given by Laurent and Gerhardt.

Cahours (48) in 1858, having shown the relationship of glycine to acetic acid (see glycine), followed the same line of reasoning and suggested that glycine, alanine, and leucine were amino acids of the fatty acid series analogous to the aminobenzoic acid series of compounds.   Accordingly, glycine was aminoacetic acid, alanine was aminopropionic acid, and leucine was aminocaproic acid.   The only error in this classification was due to the lack of knowledge of the correct structure of the carbon chain of leucine.

Further progress was made by Hüfner (53) who heated leucine with hydriodic acid in a sealed tube and obtained caproic acid and ammonium iodide.   The question still remained as to whether the acid so obtained was identical with or was merely an isomeric form of the caproic acid found elsewhere in nature.   To elucidate the question as to the relationship of caproic acid to leucine, Hüfner (54), in 1870, undertook the synthesis of leucine from commercial caproic acid.   He prepared bromocaproic acid and treated this compound with ammonia.   The solubility of the synthetic amino acid was about the same as that of the natural product.   Another synthesis was carried out in accordance with Strecker's cyanohydrin method, a procedure by which, as early as 1855, Limpricht (58) had synthesized leucine from valeraldehyde ammonia and hydrocyanic acid.   Hüfner now had three leucine preparations, one isolated from protein, the second prepared from caproic acid, and the third synthesized from valeric aldehyde.   These differed slightly from each other; "Allerdings ist die Möglichkeit, dass diese geringen Differenzen ebenso durch

die Isomerie der in den dreien enthaltenen Amyle bedingt sein können, nicht völlig von der Hand zu weisen; allein nichts desto weniger möchte ich doch, eben um der Geringfügigkeit jener Differenzen willen, alle drei auf verschiedene Weise gebildeten Leucine lieber als identische Körper, wie als blosse isomere Verbindungen bezeichnen."

Although the work of Schützenberger (63) advanced our knowledge of the chemistry of amino acids very little, it is illustrative of the difficulties that confronted chemists in separating the constituents of a protein hydrolysate even as late as in 1879. He relied entirely upon fractional crystallization to separate the products of hydrolysis of proteins from each other, a method that has since been shown to be only partially effective. Compounds of the series $C_nH_{2n+1}NO_2$ were called leucines, compounds of the series $C_nH_{2n-1}NO_2$ were called leuceines; tyroleucine was a substance of the formula $C_7H_{11}NO_2$; alanine was termed *leucine propionique*.

The constitution of leucine was finally established about 1891 by Schulze and Likiernik (62). They set up the following criteria upon which to base a comparison of the synthetic product with leucine isolated from protein. (a) The solubility of the two products must be identical. Since synthetic leucine was racemic it was necessary to determine the solubility of the racemized natural product. Schulze and Bosshard (61) had shown that complete racemization occurred after heating leucine with barium hydroxide to 170°. (b) Identical products should be obtained by means of *Penicillium glaucum* from synthetic and from racemized natural leucine. (c) On treatment with nitrous acid, the synthetic and the natural leucine should yield the same hydroxy acid. It was found that synthetic α-aminoisobutylacetic acid satisfied these criteria, while leucine synthesized from caproic acid differed from racemic leucine prepared from protein.

Other syntheses have more recently been carried out by Erlenmeyer and Kunlin (49), Fischer and Schmitz (50), and Bouveault and Locquin (44). Fischer and Warburg (51) showed that it was possible to resolve racemic leucine into its optically active components by means of the formyl compounds. The separation

of leucine and tyrosine by taking advantage of the difference in the solubility of the two substances in glacial acetic acid was described by Habermann and Ehrenfeld in 1902 (52).

GLYCINE

$$CH_2(NH_2)-COOH$$

The discovery of glycine by Henri Braconnot (68) in 1820 is the first instance in which a pure amino acid was obtained from a protein by acid hydrolysis.   Braconnot was interested in substances which, on acid hydrolysis, yielded sugar.   He had already shown that sugar could be obtained from wood, bark, straw, and hemp by this procedure and it was natural that he should attempt to see if animal substances yield similar products.   He therefore boiled gelatin with sulfuric acid for 5 hours, neutralized the acid with calcium carbonate, evaporated the filtrate from the calcium sulfate to a sirup and left it to stand for about a month.   At the end of this time he noted that crystals, which adhered to the wall of the glass vessel, had formed.   The crystals possessed a sweet taste.   It appeared to him that the product was indeed a sugar and he therefore named it *sucre de gélatine*.   This was translated into German as *Leimzucker*.   The statement, "Nous allons examiner les propriétés de ce sucre, qui pourrait à la rigueur constituer un genre nouveau si l'on ne craignait de trop les multiplier" leaves no doubt as to Braconnot's idea of the new substance.

He observed that "sucre de gélatine" was more easily crystallizable than cane sugar, that it was about as sweet as glucose, that it was about as soluble in water as milk sugar and that it was not fermentable.   On treating it with nitric acid he obtained a crystallizable product which he termed *acide nitrosaccharique*. He noted the physical properties of the new compound but carried out no further chemical work.   He did not discover that it contained nitrogen.   It appeared to him that "cette transformation est opérée par une soustraction d'hydrogène et d'azote dans les proportions nécessaires pour faire l'ammoniaque, et probablement par une absorption d'oxigène de l'acide sulfurique."

In 1838 Mulder (76) showed that both glycine and leucine could be obtained by hydrolyzing gelatin with potassium hydroxide, and that meat likewise yielded these two amino acids. Mulder analyzed his glycine with results that suggested the formula $C_8H_{18}N_4O_7$. The preparation, on being heated with lead oxide at 100°, lost 12.5 per cent of its weight, although in the absence of lead oxide it lost no weight when heated to 110°. He therefore concluded that it contained chemically bound water and, by allowing for this, the formula was reduced to $C_8H_{14}N_4O_5$ with a molecular weight of 1552.925. The compound was considered to contain two hydrogen atoms replaceable by base. Mulder's formula was gravely in error and the work of Boussingault (66, 67) at about the same time was even less accurate. In a later publication Mulder (77) stated that his original product, as well as those prepared by a number of other investigators, was probably contaminated with leucine. He now (1846) ascribed the composition $C_8H_{10}N_2O_8$ to glycine; translated into modern atomic weights this agrees with twice the present accepted formula. Mulder was not, however, the first to determine the correct formula of glycine.

Dessaignes (72) showed in 1845 that acid hydrolysis of the hippuric acid, which Ure had shown to be excreted in the urine when benzoic acid was ingested, yielded glycine. From the formulas of hippuric acid and of benzoic acid he predicted the formula of glycine, a prediction which had apparently also been made by Gerhardt. There was uncertainty, however, as to whether or not a molecule of water was lost in the cleavage of the hippuric acid. Dessaignes states, "Je serais plus porté a croire qu'au reste $C_4H_6N_2O_2$, il faut ajouter 2 équivalents d'eau, et que le véritable équivalent du sucre de gelatine est $C_4H_{10}N_2O_2$, comme l'a indiqué M. Gerhardt; mais je n'ai pas encore de preuve à apporter en faveur de cette manière de voir." But Dessaignes did not make an analysis of gycine!

Controversies were exceedingly spirited in the early days of chemistry and at times reached the height of sarcasm, as the following quotation from Gerhardt's (73) paper published in 1846 shows. "MM. Boussingault et Mulder avaient attribué au sucre

de gelatine des formules assez compliquées. Je rejette ces formules, les déclare erronées et y substitue les rapports $C_2H_5NO_2$. Nouvelle déception, nouvel arbitraire! M. Mulder reprend ses analyses, trouve qu'un mélange de leucine lui avait d'abord donné trop 'de carbone, et, par de nouvelles expériences, arrive exactement aux rapports proposés par moi, mais naturellement il ne me cite pas non plus."

The experimental data in support of the correct composition of glycine were supplied in 1846 from three different sources—Horsford (74), Laurent (75), and Mulder (77). Horsford's work was carried out in Liebig's laboratory in Giessen. It was he who suggested the term glycocoll in place of "Leimzucker." In a footnote to his article he writes "Für den wasserfreien Körper werde ich den schon vorgeschlagenen Namen *Glycocoll* gebrauchen. Wie unpassend der Name Zucker ist, hat schon Dessaignes angeführt, da er den süssen Geschmack mit vielen andern Körpern, die niemand Zucker nennt, theilt und nicht gährungsfähig ist." Two years later, Berzelius (65) suggested that this term be changed to glycine. "Dieser Name (Glycocoll) ist nicht wohlklingend und hat ausserdem den Fehler, dass er nicht mit den Namen der übrigen Basen harmonirt. Er ist zusammengesetzt aus γλυχύς, süss, und κόλλα, Leim. Da diese organische Base die einzige ist, welche süss schmeckt, so kann sie viel kürzer Glycin genannt werden, und diesen Namen werde ich anwenden."

Horsford's analysis of glycine prepared from hippuric acid gave results in accordance with the formula $C_4H_4NO_2HO$ which, in modern atomic weights, is equivalent to $C_2H_5NO_2$, the correct formula. He further showed that glycine combined both with acids and with bases and prepared a series of these compounds. The question which concerned him is expressed in his statement, "Es drängt sich nun von selbst die Frage auf, in welche der gewöhnlichen Abteilungen der Chemie soll man das Glycocoll stellen? Ist es eine Basis, eine Säure oder ein Salz?" His answer, "Man muss nach dem hier Vorgebrachten schliessen, das Glycocoll zu gleicher Zeit Säure, Basis und Salz sein kann, indem es alle Eigenschaften zeigt, wodurch jede dieser Klassen von den andern sich unterscheidet. Durch diesen Besitz so verschiedenartiger

Eigenschaften zeichnet sich dieser Körper vor allen anderen aus," clearly shows that his ideas agree with the present day conception of amphoteric electrolytes. This idea was, however, not entirely new. As early as 1810 Wollaston (79) had expressed similar views with respect to cystine.

The next step in the chemistry of glycine was the elucidation of its structure; Cahours (69) was probably the first to guess this correctly. He reasoned that, since aminobenzoic acid is formed from nitrobenzoic acid by reduction, nitroacetic acid on similar treatment should yield glycine; but nitroacetic acid had not been synthesized, so it was not possible to test this hypothesis experimentally. Later, however, he (70) showed that glycine was formed when monochloroacetic acid was treated with ammonia, and that, on treating glycine with nitrous acid, glycolic acid was produced. Cahours' experiments received further support when Perkin and Duppa (78), in the same year, synthesized glycocoll by treating monobromoacetic acid with ammonia. A number of other methods for the synthesis of glycine have been proposed; the most recent consists in hydrolyzing aminoacetonitrile with hydrobromic acid (71).

ASPARTIC ACID

CH(NH$_2$)—COOH
|
CH$_2$—COOH

The history of aspartic acid really begins with the discovery of asparagine by Vauquelin and Robiquet (118) in 1806. Vauquelin had, in this work, the coöperation of a young chemist, Robiquet, whom he describes as "jeune chimiste, qui réunit la solidité du raisonnement une grande habileté dans les expériences." Robiquet (111) had previously published a paper on the composition of the asparagus plant. The discovery of asparagine came about when a quantity of the juice of this plant, which had been concentrated by evaporation, was permitted to stand for some time. Vauquelin's own words best describe the discovery. "Ayant abandonné dans mon laboratoire, pendant un voyage qu'il (Robiquet) fit, une certaine quantité de suc d'asperges, concentré

par l'évaporation, j'y observai un assez grand nombre de cristaux, parmi lesquels deux me parurent appartenir à des substances nouvelles: comme ils avaient une forme, une transparence et une saveur différentes, il me fut facile de les séparer."

The chemical nature of this substance proved to be a puzzling problem to Vauquelin and Robiquet. They found that it left no ash on ignition and that, on treatment with nitric acid, it was decomposed with the liberation of nitrogen. They did not regard it as an acid, since it did not affect turmeric paper, nor was it a salt since it had no fixed base in combination. Without reporting an exact analysis they concluded that the substance contained hydrogen, oxygen, and carbon in definite proportions and probably also nitrogen. In their own words ". . . . il y existe un principe cristallisable comme les sels, et qui n'est cependant ni acide, ni sel neutre, et dont la solution dans l'eau n'est affectée par aucun des réactifs qui sont ordinairement employés pour reconnaître la présence et la nature des sels dissous dans l'eau; et un autre principe sucré qui parait avoir de l'analogie avec la manne." Vauquelin and Robiquet did not give a name to the new product in their original paper. Dulong (88) in 1826, in describing their work, refers, however, to the substance "qu'ils ont designée sous le nom d'asparagine." . At this time the term was in general use; it is derived from ἀσπάραγος, asparagus.

Bacon (80) in 1826 found that when alcohol was added to the aqueous extract from the root of the marshmallow (Althaea officinalis) a crystalline product separated. This he considered to be a salt of malic acid with a vegetable base and he gave it the name althèine. In 1827, Plisson (106) repeated Bacon's experiments. He found that Bacon's product possessed essentially the same properties as the substance Vauquelin and Robiquet had found in asparagus and that had since received the name asparagine. "Le malate acide de M. Bacon n'est ni un sel, ni un acide, c'est une substance azotée particulière qui joint des propriétés de l'asparagine." When Plisson heated a solution of this substance with lead hydroxide, removed the lead with hydrogen sulfide and concentrated the filtrate, he obtained a crystalline acid which, after recrystallization three times from alcohol, possessed the

following properties: "Il est sous forme de petites plaques brillantes et assez semblables à celles de l'acide borique brisé; il a peu de saveur, il est beaucoup plus soluble dans l'eau chaude que dans l'eau froide qui n'en dissout que peu; il est encore moins soluble dans l'alcool et d'autant moins que celui-ci est plus concentré." Plisson gave the name aspartic acid to this substance. "Traitée par l'hydrate de plomb, cette substance azotée que je considère comme de l'asparagine, donne lieu principalement à de l'ammoniaque et à un acide nouveau que l'on pourrait nommer *asparagique*. . . . . Comme le mot *asparagique* pourrait donner à entendre que l'acide de ce nom se rencontre à l'état naturel, je crois qu'il serait mieux d'adopter celui *d'aspartique* qui rappellerait que cet acide est artificiel."

Tiedemann and Gmelin (117) gave the name *Gallenasparagin* to a substance which they isolated from ox bile by adding hydrochloric acid, concentrating the filtrate and precipitating with alcohol. Berzelius (81) commented as follows on their product: "Von dem Asparagin aus den Spargeln ist diese Substanz so wesentlich verschieden dass ich mich wundere, wie sie für dieselbe diesen Namen wählen konnten; denn die Asparaginkrystalle aus Spargeln haben, wie sie auch bemerken, andere Winkel und werden leicht von Salpetersäure zersetzt."

Blondeau and Plisson (82) demonstrated the presence of asparagine in the roots of the comfrey in 1827 and the following year Plisson (107) showed that the crystalline substance that Robiquet (112) had isolated from licorice root and named *l'agédoïte* was also asparagine.

In 1830 Plisson and Henry fils (108) published the first analyses of asparagine and of aspartic acid. The formula $C_{12}H_{12}N_2O_4$ was ascribed to asparagine and the formula $C_{14}H_{14}N_2O_7$ to aspartic acid. In order to see if the odor observed in the urines of individuals who had eaten asparagus was formed from asparagine, a large dose of this substance was ingested; the characteristic odor, however, did not appear.

The experiments of Plisson were repeated by Wittstock (119). He concluded that asparagine did not exist preformed in the roots of the marshmallow, but believed that it was merely the ammo-

nium salt of aspartic acid that was set free during the process of isolation.

Pelouze (103), in a letter to Liebig in 1833, gave the formula of asparagine, dried at 120°, as $C_8N_4H_{16}O_5$, and that of aspartic acid as $C_8N_2H_{14}O_7$. These formulas were very nearly correct; $C_8N_4H_{18}O_6$ and $C_8N_2H_{14}O_8$ are the correct formulas expressed in the atomic weights used by Pelouze. He further stated that by the action of acids or alkalis, or of steam under pressure, asparagine is converted into ammonium aspartate. Therefore ". . . . Asparagin ist spargelsäures Ammoniak weniger 1 Atom Wasser. Wir nennen deshalb das Asparagin Asparamid." He was inclined to believe that allantoin, cystic oxide (cystine) and caffeine were compounds analogous to asparagine.

These results were shortly afterwards reported in greater detail by Boutron-Charlard and Pelouze (83). They obtained asparagine by repeatedly extracting marshmallow roots with cold water, concentrating the fluid to a sirup and permitting crystallization to take place. For the preparation of aspartic acid asparagine was hydrolyzed with barium hydroxide, the barium was removed by means of sulfuric acid and the acid was permitted to crystallize. Their idea that the conversion of asparagine to ammonium aspartate represented an hydrolysis was quite clear and definite; they likened the reaction to the hydrolysis of urea to form ammonium carbonate, the hydrolysis of oxamide to yield ammonium oxalate and the hydrolysis of benzamide to form ammonium benzoate. Save for small errors in the oxygen their formulas for asparagine and for aspartic acid were correct. The products they worked with were apparently fairly pure since their aspartic acid did not possess the meat-like taste Plisson and Henry mentioned as characteristic. The experiments of Pelouze distinctly supported those of Plisson and disproved the chief contentions of Wittstock.

Shortly after the publication of this paper, Liebig (96), who had obtained his preparations from Pelouze, published an analysis of asparagine and of aspartic acid. His data indicated that the formula of asparagine is $C_8H_{16}N_4O_6$. In the crystalline state the product contained two molecules of water. To aspartic acid he

assigned the formula $C_8H_{10}N_2O_6$.[10] Liebig pointed out that, since the formula of asparagine was obtained by the addition of two formula weights of ammonia to one of aspartic acid, the conversion of asparagine to aspartic acid and ammonia should proceed without the entrance of water into the reaction. On the basis of this reasoning he concluded that "das Asparamid zu der Klasse von Amiden nicht gerechnet werden darf, sondern es gehört einer andern an, wo eine wasserfreie Sauerstoffsäure mit Ammoniak zu einem Körper verbunden ist, der mit Ammoniaksalzen keine Aehnlichkeit besitzt, obgleich er im crystallisirten Zustande genau die Menge Wasser enthält, welche dem Atomverhältniss des Wassers in den Ammoniaksalzen entspricht, die durch Sauerstoffsäuren gebildet werden, allein dieses Wasser kann durch Wärme daraus entfernt werden, ohne dass die Verbindung selbst geändert wird." Liebig was therefore led to question the view of Dumas that urea might be considered as an amide of carbon monoxide and suggested instead that it was formed from cyanic acid and ammonia and that its structure was entirely like that of asparagine. Liebig proposed that "man wird also vorläufig dem Asparamid seinen älteren Namen Asparagin wieder geben müssen."

This view, founded upon an erroneous analysis of aspartic acid and backed by the weight of Liebig's prestige, led, as will be seen, to some interesting developments. Under the date of May 30, 1833, Liebig (99) wrote to Berzelius, "Ich habe kürzlich die Asparaginsäure und das Asparamid analysirt, ich habe eine andere Zusammensetzung erhalten wie Pelouze aber sie ist so, dass die Theorie der Verwandlung des Asparamids in Asparaginsäures Ammoniak ganz so bleibt wie sie von Pelouze hingestellt worden ist. Asparamid ist $C_4N_2H_8O_3$, Asparaginsäure 2 $C_4NH_7O_4$, ich verbürge aber diese Resultate noch nicht ganz, ich habe sie Pelouze mitgetheilt um ihn zu veranlassen seine Arbeit zu wiederholen, er schreibt mir aber dass seine neuste Analyse mit seiner frühren übereinstimmt, und er will mir nun nochmals sehr reines Aspara-

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[10] This formula was expressed in this doubled form so as to conform to the even number rule. The formula of asparagine was written so as to agree with that of aspartic acid.

gin und A. säure zur Analyse schicken." In 1838 Liebig (97) published an analysis of aspartic acid which he had obtained by hydrolyzing asparagine with potassium hydroxide. The data indicated that the formula of aspartic acid was $C_8H_{14}N_2O_8$. This, divided by two, represents the present accepted composition of aspartic acid.

The next step lay in the elucidation of the structure of asparagine and of aspartic acid. The first work on this subject was carried out by Piria (104) at the University of Pisa. He showed that asparagine could be obtained from a number of sources, particularly from vetch seedlings. On treating asparagine with copper oxide he obtained the crystalline copper salt. When subjected to bacterial fermentation asparagine was converted into ammonium succinate. Contrary to the statement of Liebig (98) that "l'acide aspartique lui-même, soumis a l'ébullition avec de l'acide chlorhydrique concentré, ou fondu avec de la potasse caustique, se transforme en ammoniaque et en un nouvel acide très-soluble dans l'eau et non étudié encore," Piria was able to show that asparagine, when boiled either with hydrochloric acid or with nitric acid free from nitrous acid, was converted into aspartic acid and that this substance was not decomposed by these acids. Alkaline fusion of asparagine led to the formation of acetic and oxalic acids. A very important observation made by Piria was that asparagine and aspartic acid, on treatment with nitrous acid, were converted into malic acid with loss of nitrogen. He concluded that "ces deux corps comme deux amides de l'acide malique correspondant à l'oxamide et l'acide oxamique, qui sont les amides de l'acide oxalique." Piria did not, however, realize the possibility that the two amides of malic acid were isomeric with aspartic acid and asparagine. Owing to his erroneous explanation of the transformation of the latter substances into malic acid, the chemistry of aspartic acid and asparagine was led astray.

Shortly after the publication of Piria's work, Dessaignes (87) announced that it was possible to convert the ammonium salts of malic, maleic, and fumaric acids into aspartic acid by heating. He showed further that aspartic acid was converted, by bacterial

action, into succinic acid. The work of Dessaignes attracted the attention of Pasteur (101), particularly since Dessaignes' synthesis of aspartic acid presented certain problems in the field of optical activity. Pasteur had shown that both malic acid and aspartic acid possessed the property of rotating the plane of polarized light, while fumaric acid did not. If Dessaignes' synthesis was correct it apparently involved the formation of optically active aspartic acid from inactive fumaric acid. But Dessaignes' synthetic aspartic acid proved to be optically inactive; this led Pasteur to a study of the chemical and optical properties of active and inactive aspartic and malic acids. He compared the malic acid that had been obtained from aspartic acid with the natural product with the result that "je m'assure que l'acide malique, ainsi obtenu, était en tout point identique, sous le triple point de vue chimique, cristallographique et optique, avec l'acide malique du sorbier, des pommes, des raisins, et du tabac."

In a subsequent communication Pasteur (102) made the very important observations that the diamide of malic acid, malamide, obtained by synthesis from malic acid ester and ammonia was isomeric but not identical with asparagine. It was also known that, whereas oxamide and oxaminic acid, when heated with alkali, yielded all of their nitrogen in the form of ammonia, asparagine, when similarly treated, yielded only one-half of its nitrogen in this form and aspartic acid yielded none at all. This at once reopened the question of the constitution of aspartic acid and of asparagine.

It was evident from the behavior toward alkali that the nitrogen in asparagine was present in two forms. Kolbe pointed out (94) that aspartic acid was an amino acid and a derivative of succinic acid, not of malic acid, while asparagine was the amide of aminosuccinic acid. He regarded aspartic acid as a dibasic acid, "dass sie von den Alkalien nicht zwei Atome zu sättigen vermag, ist bei ihren sehr schwach saueren Eigenschaften wenig befremdend. Gleichwie das Glycocoll, Alanin, Leucin und gar das Taurin, überhaupt die Amidosäuren von den primären Säuren dadurch in bemerkenswerther Weise sich unterscheiden, dass sie kaum noch als Säuren anzusprechen sind, und grösstentheils

sogar basische Eigenschaften haben, so ist auch bei der Asparaginsäure der sauere Character der Bernsteinsäure durch den Eintritt von Amid für Wasserstoff in dem Grade abgeschwächt, dass sie eben sowohl mit Säuren wie mit Basen chemische Verbindungen eingeht."

Although both Dessaignes (87) and Engel (90) had synthesized aspartic acid, their procedures did not throw any light upon the structure of this substance. It remained for Piutti (105) in 1887 to prove by synthesis the correct structure of asparagine. Ethyl oxalate and ethyl acetate, in the presence of sodium alcoholate, react to give the sodium salt of oxalacetic ester. This was converted into the oxime by treatment with hydroxylamine. On reduction with sodium amalgam the oxime was converted into aspartic acid. A second synthesis was carried out by Schmidt and Widmann (115). By treating acetylsuccinic acid ester with nitrous acid it was converted into a nitroso derivative which, on reduction, yielded aspartic acid.

The procedures of Schiff (114) or of Pachlopnik (100) are also employed for the preparation of aspartic acid; both methods use asparagine as the starting material. As early as 1876 Guareschi (93) studied the solubility of asparagine and aspartic acid and prepared certain uramino compounds. Fischer and Koenigs (91) synthesized a number of peptides containing aspartic acid.

Ritthausen (109) was the first to isolate aspartic acid from the products of protein hydrolysis. He worked with conglutin and legumin. After separating tyrosin and leucine he permitted the acidified solution to stand over sulfuric acid. A considerable quantity of white material separated which, when removed and recrystallized, yielded glutamic acid. The mother liquor deposited nothing further of a definite nature even when treated with alcohol and ether; its strongly acid reaction suggested, however, that some acidic substance was present and Ritthausen therefore added barium carbonate which dissolved with effervescence. After filtration from the excess of reagent the fluid was treated with alcohol. This step is of the greatest historical significance as will shortly appear. The result of this treatment

is best given in Ritthausen's own words. "Es entstand hierbei ein schmieriger seidenglänzender, bald zu zäher Masse erstarrender Niederschlag, der, wiederholt aufgelöst und gefällt, das Barytsalz, einer neuen stickstoffhaltigen, ohne Zweifel wohl einer Aminsäure, darstellt, und mit dem aus Conglutin der Lupinen in gleicher Weise erhaltenen Körper identisch ist." He was misled by the analysis as to the nature of the amino acid. He concluded "dass die Säure nicht Succinaminsäure ist sondern eine Aminsäure von der Zusammensetzung $C_8H_{14}N_2O_6$." He named the product *Legaminsäure* in the words ". . . . sollte späteren Untersuchungen sicher dargethan werden, dass die Säure eine eigenthümliche Substanz ist, würde ich ihr den Namen *Legaminsäure* beilegen."

In 1869 Ritthausen (110) published the results of further investigation of this substance. The material secured by the precipitation by means of alcohol of the calcium salts of the acids was found to be a mixture of two crystalline acids together with a noncrystalline sirup of acid reaction. The crystalline acids could be separated by means of 50–60 per cent alcohol in which a part was insoluble. This separated in rhombic prisms, or sometimes in leaflets, when recrystallized, and was found to be identical with aspartic acid. The more soluble crystalline acid was glutamic acid. The copper salt was found to be especially advantageous for the separation of small amounts of aspartic acid from the mother liquor, and this salt is still the most useful known compound of aspartic acid for isolation purposes. Aspartic and glutamic acids were recognized to be homologous compounds; the former yielded malic acid, the latter glutanic acid, on treatment with nitrous acid.

There are two points of the greatest significance in these papers. Ritthausen discovered aspartic acid as a result of precipitating its barium salt, and later its calcium salt, by means of alcohol. This method of dealing with the dibasic amino acids was independently rediscovered by Foreman (92) in 1914 and was later employed by Dakin (85). Neither Ritthausen nor the later investigators can, however, lay any claim to using the method

for the first time. It is at least as old as Scheele (113) and in the hands of this extraordinarily resourceful worker led to the discovery of malic acid. Curiously enough, however, the great value of the method was not emphasized either by Scheele nor, later, by Ritthausen and, save in some early work by Schulze (116) on the analysis of proteins, it was entirely overlooked until Foreman and Dakin developed it into a standard method of protein analysis.

The second point is the nature of the acid in the sirupy mother liquor of the glutamic acid described by Ritthausen. Glutamic acid is largely converted into the very soluble pyrrolidonecarboxylic acid when its solutions are boiled with water. Ritthausen worked over his mother liquors for several months and secured about 8 grams of relatively pure glutamic acid from them. He recorded no suspicion that the glutamic acid might be undergoing change and there is every probability that an appreciable part of the glutamic acid present was, in fact, converted to pyrrolidonecarboxylic acid during his manipulations. But there is another highly significant possibility. Oxyglutamic acid is also extremely soluble and it is by no means improbable that this substance was likewise present. The properties of this acid are such, however, that there is little wonder Ritthausen failed to isolate it.

Ritthausen showed that aspartic acid was a constituent of vegetable proteins; Kreusler (95) extended the observations to animal proteins, and found it in casein and in egg proteins. It has since been observed to be widely if not universally distributed.

A number of syntheses of aspartic acid have been described. A recent one by Dunn and Smart (89) is of interest because of the novel conditions employed.

Hydroxyaspartic acid has been synthesized by Dakin (86). The product consists of inactive *para-* and *anti*-hydroxyaspartic acid but this substance has not yet been shown to occur in nature. Chibnall and Cannan (84) have extended Dakin's work by synthesizing hydroxy asparagine and measuring the dissociation constants. This product also has as yet not been found in nature.

TYROSINE

HO⟨◯⟩CH₂—CH(NH₂)—COOH

Tyrosine was discovered by Liebig (142) in 1846 in the course of an investigation of the nature of the products that proteins yield on decomposition with alkali. He fused crude casein with an equal weight of potassium hydroxide, dissolved the mass in hot water, acidified with acetic acid and permitted the solution to cool, ". . . . so scheidet sich eine Masse von sehr feinen Nadeln ab, welche in kaltem Wasser sehr schwer, in Alkohol und Aether unlöslich sind. Durch wiederholtes Auflösen in Wasser, dem man etwas kohlensaures Kali zusetzt und Fällung mit Essigsäure, erhält man diesen Körper rein weiss in seidenglänzenden Nadeln." Liebig's preliminary analysis suggested the formula $C_{16}NH_9O_5$ for the new substance but it is not certain that he regarded it as an amino acid, although some indication that the compound possessed amphoteric properties is given in the sentence "Der Körper, obwohl in Alkalien leicht löslich verbindet sich mit Säuren." He made no attempt to determine its structure. In a later paper (143) he mentioned that the same substance could be obtained from fibrin and from serum albumin, and gave the name tyrosine (τυρός, cheese) to it in the words ". . . . ein krystallinischer Körper, das Tyrosin (mit welchem Namen ich den Bd. LVII S. 127 der Annalen beschriebenen Körper belegt habe. . . . ."

de La Rue (129) obtained tyrosine in 1848 during an investigation on the composition of the cochineal insect. Carminic acid was removed from the aqueous extract with lead nitrate, and the filtrate, after removal of the lead, was concentrated to a sirup; a white crystalline product separated. This was recrystallized several times from hot water. An analysis indicated that the formula was $C_{18}H_{11}NO_6$ ($C_9H_{11}NO_3$ in modern atomic weights) which is the correct formula for tyrosine. Comparison of his product with a specimen of the substance Liebig had obtained from casein showed that they were identical. He answered the question whether tyrosine occurred in the free state, or was set

free in the course of the isolation, by the statement, "Man kann also annehmen, dass dieser Körper in dem getrockneten Insekte fertig gebildet enthalten ist."

Bopp (127) in 1849, in Liebig's laboratory, investigated the conditions under which tyrosine could best be obtained from casein, fibrin, and serum albumin.   He found that it was very difficult so to control the conditions of the potassium hydroxide fusion as to obtain good yields of tyrosine.   He noted, however, that both tyrosine and leucine were not destroyed by long continued boiling with hydrochloric or diluted sulfuric acid.   Braconnot had obtained leucine from several proteins by boiling them with sulfuric acid but Mulder had stated that, if hydrochloric acid were used, only ammonia and the ammonium salt of humic acid were produced.   This seemed to require verification.   Bopp therefore treated casein with several times its weight of hot concentrated hydrochloric acid; the protein dissolved with the formation of an intense violet color that slowly turned brown as the heating was continued.   After boiling the mixture for 6 to 8 hours the decomposition had proceeded far enough to permit the isolation of tyrosine and leucine.   This experiment is of considerable historical importance; it is the first recorded successful hydrolysis of a protein by hydrochloric acid.   Bopp used a mixture of sulfuric and hydrochloric acids in his main experiments.   The reagents were removed by adding an excess of calcium carbonate and heating to expel ammonia; the precipitate was removed, a small excess of sulfuric acid was added to throw down the calcium, the excess of this and most of the chloride were removed by adding lead oxide; finally the excess of lead was precipitated by hydrogen sulfide.   The filtrate, when evaporated, yielded leucine and tyrosine which were separated from the sirupy mother liquor by dilution with 80 per cent alcohol.   Bopp did not regard it as necessary to identify his products by analysis; the crystalline form and behavior left him in no doubt of their identity and, indeed, his description of the tyrosine obtained by acid hydrolysis, and his comparison with the preparations secured from alkali fusion, leave no question that Bopp was the first to prepare tyrosine from acid hydrolysates.   Hinterberger (139), also working in

Liebig's laboratory at about the same time, prepared tyrosine by sulfuric acid hydrolysis of horn; analysis of his product led to the correct formula $C_{18}H_{11}NO_6$ already given by de La Rue.   His preparation was also identical with those of Liebig and of Bopp.

Müller (144), Leyer and Köller (141), Piria (147), Wicke (154), and Städeler (150, 151) during the next decade demonstrated the presence of tyrosine, together with leucine, in a wide variety of protein substances which included hair, feathers, gliadin, and silk fibroin.   Their work definitely established the fact that tyrosine belonged to the group of substances which includes leucine and glycine.   For example, Wicke wrote, "Das gleichzeitige Auftreten des Tyrosins und Leucins bei Zersetzung der Proteinsubstanzen, die einigermassen ähnlichen Formeln und leicht Löslichkeit beider in Säuren und Alkalien lassen vermuthen, dass das Tyrosin eine ähnliche Constitution wie das Leucin besitzt und vielleicht zur Reihe der aromatischen Säuren in demselben Verhältniss steht wie das Leucin zur Reihe der fetten Säuren."

The presence of tyrosine, and also of leucine, was demonstrated in tissues; Chevallier and Lassaigne (128) found wart-like, white round bodies of the size of poppy seeds in a cadaver.   This substance was called by them *tubercules cystinoïdes* or *xanthoprotein*. No analysis was reported.   They considered that the properties of this substance were intermediate between those of cystine and xanthine; the description, however, indicates that they were dealing with tyrosine.   Frerichs and Städeler (135) were the first to report the presence of tyrosine and leucine in a diseased liver. Later (136) they demonstrated the presence of these amino acids in a number of other organs as well as in blood and urine.   In 1860 Neukomm (145) reported the presence of both leucine and tyrosine in the organs of human cadavers in a variety of pathological conditions.   The relation of tyrosine to homogentisic acid, a substance found in the urine in alcaptonuria, was first studied by Bödeker (126).   It has been the subject of extensive study since that time (137).

Städeler (151) was the most thorough of the early workers who investigated the behavior of tyrosine.   He prepared a series of compounds with bases, acids and heavy metals and a similar

series of compounds of mono- and dinitro-tyrosine as well. He showed that it was possible to convert tyrosine into chloranil ($C_6Cl_4O_2$), and recognized the importance of this reaction in throwing light upon the structure of tyrosine. "Von den mitgetheilten Zersetzungen des Tyrosins scheint vorläufig diese letztere allein geeignet zu sein, einiges Licht auf die Constitution desselben zu werfen." He emphasized the point that tyrosine belongs to the group of compounds that includes glycine and leucine. "Ohne Zweifel hat das Tyrosin eine ähnliche Constitution wie diese Körper; es verbindet sich ebenfalls nicht nur mit Basen, sondern auch mit stärkeren Säuren, und zwar nach Art des Ammoniaks." He noticed also that tyrosine is a dibasic acid ("tritt das Tyrosin als schwache zweibasische Säure auf").

Schmitt and Nasse in 1865 (149) concluded that tyrosine is related to salicylic acid. They based this view on these facts: (a) that tyrosine could be converted into chloranil; (b) that on dry distillation it yielded phenylalcohol; (c) that like salicylic acid it gave a colored compound with ferric chloride; (d) that it was a dibasic acid. They assumed that tyrosine was ethylaminosalicylic acid, and considered that it bore a relationship to salicylic acid similar to that which sarcosine bears to acetic acid. They were, however, unable to synthesize tyrosine by treating chloro- or iodo-salicylic acid with ethylamine. On heating aminosalicylic acid, carbon dioxide is split off and oxyphenylamine formed; Schmitt and Nasse therefore thought that, if tyrosine were ethylaminosalicylic acid, it should under similar conditions lose carbon dioxide and form ethyloxyphenylamine. Schmitt and Nasse did obtain a substance of the composition of the compound they expected; they did not, however, prove its structure nor consider the possibility that the carbon dioxide might be split off from a side chain.

The assumption of Schmitt and Nasse was disproved by Barth (122) when he showed that, on alkaline fusion, tyrosine yields acetic acid and p-oxybenzoic acid. From this evidence Barth assumed that tyrosine was the ethylamino derivative of p-oxybenzoic acid. In a later communication, Barth (123) corrected this assumption by showing that such substances as aminobenzoic

acid, amino-*p*-oxybenzoic acid, aminohydrocinnamic acid and aminosalicylic acid, on alkaline fusion, do not yield *p*-oxybenzoic acid but give non-crystalline products. This showed that the amino group was not replaced by hydrogen as should be the case if *p*-oxybenzoic acid were formed. Confronted with the fact that alkaline fusion of tyrosine gave almost quantitative yields of *p*-oxybenzoic acid, Barth made a second guess as to the structure of tyrosine and this later proved to be correct, ". . . . so erscheint es am wahrscheinlichsten, dass das Tyrosin als eine Oxy-phenylamidopropionsäure zu betrachten ist." This view was in harmony with the observation that ammonia is set free when tyrosine is treated with hydriodic acid. Barth, moreover, showed that the product obtained by Schmitt and Nasse on carefully heating tyrosine was neither amidophlorol nor ethylaminooxy-benzoic acid as the Schmitt and Nasse hypothesis would require, but a compound in which the amino group was contained in the side chain.

Ost (146) was able to confirm Barth's experiments on the alkali fusion of tyrosine and related compounds, but his attempt (124) to synthesize tyrosine by preparing oxyphenylchloroacetic acid and treating this with ammonia failed. Further proof of the structure of tyrosine as suggested by Barth was furnished by Baumann (125) when he showed that tyrosine yields *p*-oxyphenyl-propionic acid on bacterial decomposition.

The synthesis of tyrosine was first accomplished by Erlenmeyer and Lipp (133) who treated *p*-aminophenylalanine with nitrous acid. A second synthesis was carried out by Erlenmeyer, Jr. and Halsey (132). They condensed hippuric acid with *p*-oxy-benzaldehyde in the presence of acetic anhydride and sodium acetate to form the lactimid. On alkaline hydrolysis, *p*-oxy-*α*-benzoylaminocinnamic acid was formed which, on reduction with sodium amalgam, yielded benzoyltyrosine; hydrolysis of this yielded tyrosine. This synthesis is essentially the same as that used by Erlenmeyer, Jr. (131) for the preparation of phenyl-alanine. Wheeler and Hoffman (153) synthesized tyrosine by boiling anisalhydantoin with hydriodic acid and red phosphorus. In this reaction reduction of the double bond takes place, ammo-

nium iodide is set free, the hydantoin ring is opened and the urea grouping undergoes hydrolysis all in one operation. The yield of tyrosine is practically quantitative. Sasaki's (148) method consists in condensing glycine anhydride with anisaldehyde to form the diketopiperazine. On boiling with hydriodic acid and red phosphorus, reduction and hydrolysis take place yielding tyrosine to the extent of about 90 per cent.

The separation of leucine from tyrosine proved to be a stumbling block to many of the earlier workers; this was accomplished by Habermann and Ehrenfeld (138) by the use of glacial acetic acid, which dissolves leucine but not tyrosine. Suzuki (152) separated these amino acid by fractional recrystallization of the hydrochlorides.

Emil Fischer (134) was the first to separate the optical isomers of synthetic tyrosine. This was done by crystallization of the brucine or cinchonine salts of benzoyltyrosine. Abderhalden and Sickel (121) obtained d-tyrosine by the action of pancreatic juice on the racemic ethyl ester of tyrosine. They resolved the racemic mixture by means of the brucine salts of the formyl derivative. Finally, Ehrlich (130) accomplished the same thing by means of yeast in the presence of sugar.

On decarboxylation, tyrosine yields tyramine, a substance which physiologically is very potent. This decarboxylation was accomplished in the laboratory by Johnson and Daschavsky (140) by heating tyrosine with a mixture of diphenylmethane and diphenylamine. Abderhalden and Gebelein (120) later used diphenylamine alone for this purpose.

ALANINE

$$CH_3—CH(NH_2)—COOH$$

Alanine is one of two protein amino acids obtained by synthesis before being shown to be products of the hydrolysis of proteins; its discovery by Strecker (157) in 1850 is the basis of the now widely used cyanohydrin reaction. Liebig had shown that lactic acid, on oxidation, yields acetaldehyde. Strecker thought that it should be possible to synthesize lactic acid from aldehyde and formic acid. His reason for advancing this hypothesis was that

mandelic acid, on oxidation, yields benzaldehyde.   Mandelic acid is formed by treating benzaldehyde with a mixture of hydrocyanic and hydrochloric acids, and it was supposed that the reaction involved the action of nascent formic acid on benzaldehyde.   In reality, however, benzaldehyde cyanohydrin is formed which, on hydrolysis, yields mandelic acid.

Strecker obtained lactic acid but not in the expected way.   He treated aldehyde ammonia with hydrocyanic acid in the presence of an excess of hydrochloric acid; on concentrating the solution ammonium chloride separated.   Strecker's own words best describe his discovery, ". . . . und es bleibt eine stark saure, dicke Mutterlauge, welche die salzsäure Verbindung eines neuen Körpers enthält, den ich *Alanin* nennen will."   Hydrochloric acid was removed as lead chloride and the excess of lead by means of hydrogen sulfide; the new substance crystallized when this solution was concentrated.   In coining the word alanine Strecker used the first syllable of the word aldehyde in order to denote its origin.

Strecker noted that alanine combines both with acids and with alkalies and forms salts with the heavy metals.   He classified it in a series of homologous compounds that included glycine as its first and leucine as its fifth member.   On treating alanine with nitrous acid, lactic acid was obtained.

In 1875 Schützenberger and Bourgeois (156) subjected silk to the action of barium hydroxide in an autoclave at 150–200°. Fractional crystallization of the resulting amino acids yielded, according to their statement, 10 per cent of tyrosine, 60 per cent of a mixture of equal parts of glycine and alanine, 10 per cent of aminobutyric acid and 20 per cent of an amino acid of the acrylic acid series.   None of the products was rigidly identified and no analyses were given.   A few years later Schützenberger (155) published a long paper on the products of hydrolysis of egg albumin under similar experimental conditions.   He separated the various substances by fractional crystallization and followed the composition of the fractions by carbon, hydrogen, and nitrogen determinations. ' An analysis of one of the fractions gave figures that agreed, within the conventional 0.2 per cent, with the theo-

retical composition of alanine. No other tests were described nor were any characteristic salts prepared. Although he does not refer to Strecker's synthetic alanine, the inference is that he was acquainted with it. This is borne out by his statement, "Parmi les composés amidés homologues de la leucine, j'ai encore rencontré, mais en petites quantités seulement, *l'alanine* $C_3H_7AzO_2$, ou leucine propionique; elle a été caractérisée par l'apparance de ses cristaux et par sa composition centisimale."

Schützenberger's claim to the discovery of alanine among the products of hydrolysis of proteins rests, then, upon a single analysis of a crystalline fraction but is unsupported by any other evidence. He may equally well be regarded as the discoverer of aminobutyric acid, although no subsequent worker has yet succeeded in identifying this amino acid among the products of hydrolysis of proteins.

In 1888 Weyl (158) isolated alanine from the hydrolytic cleavage products of silk fibroin. After removal of the tyrosine and evaporation of the solution a quantity of substance equivalent to 15 per cent of the protein crystallized out in a manner recalling the behavior of "leucine," that is to say, it separated in nodular masses. Cramer, in 1865, had recorded a similar experience, but unfortunately omitted to purify the material. Weyl recrystallized his substance from dilute alcohol with the aid of a little ammonia, and secured it finally in large rhombic plates which gave results, on analysis, very close to the theoretical requirements of alanine. Furthermore the behavior on heating was precisely like that of synthetic alanine and analysis of the copper salt confirmed the identification. Weyl stated that he could find no leucine in silk, and in fact the best modern analyses indicate that less than 2 per cent is present. He suggested that alanine acts as a substitute for leucine in this unusual protein. An excerpt from his article shows that he clearly recognized alanine as α-aminopropionic acid. "Die Analyse . . . . ergab Werthe welche mit Alanin (Amidopropionsäure) . . . . stimmen. Das Alanin der Seide ist hiernach höchst wahrscheinlich α-alanine." Weyl apparently did not consider that the presence of alanine in the protein molecule had been adequately established by Schützen-

berger and Bourgeois and, with some justice, claims the credit for this for himself in the statement, "Durch vorstehende Untersuchung ist Alanin zum ersten Male mit Sicherheit als Zersetzungsproduct eines Proteïds nachgewiesen worden."

It remained for Emil Fischer and his associates to show that alanine was a widely distributed constituent of proteins.

VALINE

$$\begin{array}{c} CH_3 \\ \diagdown \\ \diagup \\ CH_3 \end{array} CH-CH(NH_2)-COOH$$

In 1856, von Gorup-Besanez (163) published a paper on the chemical constituents of certain gland extracts. Among the substances in which he was interested, and which he sought, were the amino acids, particularly leucine and tyrosine. His method was to mince the glands, extract them with water, free the fluid from coagulable protein by heating, treat with barium hydroxide to precipitate sulfates and phosphates, and finally evaporate the fluid to a sirup for crystallization. He demonstrated the presence of leucine in the thymus, thyroid, spleen, liver, and pancreas, but only in the last was he able to demonstrate the presence of tyrosine. In the pancreas, von Gorup-Besanez found a substance that was very like leucine in its behavior, yet could be separated from the latter because of its lesser solubility in boiling alcohol. "Der in kochendem Weingeist schwieriger lösliche Theil besteht im Wesentlichen aus einem dem Leucin homologen und ihm in vielen Puncten ähnlichen Körper." An analysis of the recrystallized product showed that its composition could be expressed by the formula $C_{10}H_{11}NO_4(=C_5H_{11}NO_2)$; this corresponds to the composition of valine. It was characterized by von Gorup-Besanez in the following words: "Weisse glänzende, mit freiem Auge erkennbare prismatische Krystalle, die für sich und in Flüssigkeiten betrachtet, durchsichtig sind, aber trocken und in grosserer Menge undurchsichtig erscheinen, indess keineswegs jenes blendend weisse kreideähnliche Aussehen zeigen, wie das Leucin." He classified the new substance as one of the homologous series of compounds which included glycine, alanine, and leucine, but carried out no further work on it.

Twenty-three years after the publication of von Gorup-Besanez' paper, Schützenberger (170) in an extensive investigation on the constituents of albumin, reported the presence of aminovaleric acid among the cleavage products. "Nous verrons plus loin que l'acide amidovalérique ou butalanine existe dans les cristallisations A; sa présence expliquerait pourquoi le carbone a été trouvé souvent intermédiaire entre 5 et 6 pour 1 atome d'azote, mais elle ne rend pas compte de l'abaissement de l'hydrogène su-dessous du rapport C^nH^{2n+1}." The formula $C_5H_{11}AzO_2$ was assigned to his butalanine and there is little doubt that he was dealing with valine.

Schulze and Barbieri (169) showed in 1883 that aminovaleric acid was present, along with other amino acids, in the sprouts of *Lupinus luteus*. Their product was isolated as the copper salt after removal of phenylalanine and leucine. Later Schulze (168) also found it in the sprouts of *Vicia sativa*.

von Gorup-Besanez (163) had indicated that the substance he had isolated from the pancreas was the fifth member of the series of compounds which included glycine, alanine, and leucine, and Schützenberger (170) without definite structural proof, considered his butalanine to be aminovaleric acid. The empirical formula $C_5H_{11}NO_2$ offered several possibilities; the substance might be either amino-*n*-valeric acid, aminoisovaleric acid, or ethylmethyl-aminoacetic acid. To elucidate the structure of von Gorup-Besanez' compound, Clark and Fittig (160) in 1866 undertook the synthesis of aminovaleric acid. Cahours (159) had previously stated that aminovaleric acid (Valeraminsäure) could be synthesized by treating bromovaleric acid with ammonia but, as he did not describe the properties of his product, it is doubtful that the synthesis was actually carried out. Clark and Fittig (160) synthesized aminovaleric acid by treating bromovaleric acid with aqueous ammonia at 100° for 24 hours. They made a comparison between their synthetic product and that isolated by von Gorup-Besanez. The solubilities of the two products were similar, but there was some difference in the melting points. Despite this, Clark and Fittig were inclined to regard the products as identical and attributed the differences noted to possible im-

purities in von Gorup-Besanez' product. In a note that appeared shortly after the publication of Clark and Fittig's paper, von Gorup-Besanez (164) accepted their conclusions as to the probable identity of the synthetic and the natural products. None of the early workers, however, considered the possibility that some of the differences between their products might be due to the difference in optical activity between the synthetic product and the natural; neither did the possibility that von Gorup-Besanez' product was aminoisovaleric acid occur to them.

Schmidt and Sachtleben (167) in 1878 pointed out that the aminoisobutylformic acid (aminoisovaleric acid) synthesized by them was identical with the aminovaleric acid synthesized by Clark and Fittig, and thus showed that the product synthesized by the latter was aminoisovaleric acid and not aminovaleric acid. This view received support from Lipp (165) who synthesized α-aminoisovaleric acid from aminoisovaleronitrile and showed that it was identical with Clark and Fittig's product.

Two years later Lipp (166) took up the problem of the structure of valine. He considered that, since Clark and Fittig's product was aminoisovaleric acid and did not exhibit properties identical to von Gorup-Besanez' product, it must be either amino-n-valeric acid or ethylmethylaminoacetic acid. He synthesized the former from n-butylaldehyde ammonia. A comparison between the products of von Gorup-Besanez, of Clark and Fittig, of Schützenberger, and his own amino-n-valeric acid was now made. This showed that von Gorup-Besanez' amino acid was not identical with the two synthetic amino acids. The difficulty lay in the fact that the comparison was made from the description of his product furnished by von Gorup-Besanez and not by direct observation of the actual preparation. Moreover, the question of the effect of the optical activity on the properties of the amino acid was again not taken into account.

In 1902, Slimmer (171) published the results of his syntheses of α-aminoisovaleric acid, α-amino-n-valeric acid, α-aminomethylethylacetic acid, together with such derivatives of each as the phenylisocyanate, esters and hydantoins. The properties of each compound were carefully determined.

Previous to 1901 valine was known only as a substance occasionally found in gland or plant extracts.   Its relationship to the amino acids that result from the hydrolysis of proteins was obvious and had been noted by its discoverer, but the essential step of isolation from a protein hydrolysate became possible only after the development of the ester distillation method.   Fischer found α-aminovaleric acid in the ester fractions that distilled at 40–80° at 10 mm. and identified the substance by analysis of the free acid and of its copper salt (161).

Fischer (162) later established the fact that valine is α-aminoisovaleric acid.   He prepared the formyl derivative of synthetic *dl*-valine and resolved this into its optically active components by means of brucine.   He showed that the optical rotation of his *d*-valine was essentially the same as that reported by Schulze for valine isolated from *Lupinus luteus*, and also found that, like the two optically isomeric leucines, the *d*- and *l*-valine can be distinguished by taste.   *d*-Valine is only slightly sweet and at the same time somewhat bitter, while *l*-valine is decidedly sweet.   Fischer is responsible for the suggestion that the term valine be used to designate α-aminoisovaleric acid.   "Im Einverständniss mit Hrn. E. Schulze schlage ich dafür das Wort "Valin" vor woraus sich für das Radical $(CH_3)_2CH \cdot CH(NH_2)CO$, das in den Polypeptiden enthalten ist, die Bezeichnung "Valyl" ergiebt."

SERINE

$$CH_2(OH)—CH(NH_2)—COOH$$

Although serine is one of the most difficult of all the amino acids to isolate from proteins and is even yet an exceedingly rare substance, it was one of the early amino acids to be discovered. In 1865 Cramer (172) carried out a thorough investigation of raw silk.   He isolated the gelatin-like protein found on the surface of the fibroin, named this protein sericine and subjected a purified specimen of 6 grams of it to hydrolysis with sulfuric acid.   Concentration of the hydrolysate yielded a crop of tyrosine; "später erschienen Drusen von Erbsengrösse, die aus kleinen harten, etwas süsslich schmeckenden Krystallen zusammengesetzt

waren." A yield of approximately 10 per cent of this material was obtained. Recrystallization gave a preparation that he at first considered to be glycine; the copper content of the copper salt was, however, much too low. "In der That ergab auch die weitere Untersuchung eine durchaus abweichende Zusammensetzung. Ich werde den in Frage stehenden Körper unter dem Namen Serin beschrieben."

Larger quantities of crude sericine were hydrolyzed and more of the substance was secured. A very accurate analysis led to the correct formula $C_6H_7NO_6$, or in modern atomic weights $C_3H_7NO_3$. The new acid therefore differed from alanine by one oxygen equivalent but had many chemical properties in common with Strecker's synthetic substance. It formed soluble salts with mineral acids and with bases and the hydrochloride, nitrate, and sulfate were prepared in crystalline form.

The relation of synthetic alanine to lactic acid was known; according to its formula serine should be similarly related to glyceric acid. This substance was, in fact, obtained by Cramer by the action of nitrous acid on serine and was analyzed as its calcium salt. Cramer further drew the deduction that it should be possible to convert serine to alanine by reduction although he did not attempt the reaction; most significant of all he pointed out that cystine, then known only as a constituent of certain rare urinary calculi, was closely related to the new acid, inasmuch as cystine contains one atom of sulfur in place of one of the oxygen atoms of serine. Cramer's paper is one of the great classics of protein chemistry; for brilliance of conception and execution, for the far-reaching quality of its generalizations and for lucidity of expression it is hardly excelled.

Serine was not encountered again for more than thirty years. Fischer and Skita (181) obtained a fraction, from the amino acid esters of high boiling point derived from the hydrolysis of silk, that was extremely difficult to purify. The results of analysis suggested that oxyacids were present and they thought serine to be a probable constituent. Later (182) they found that the difficulties arose largely from the decomposition of the esters during distillation; this could be largely avoided by conducting the later

stages of the distillation at very low pressure.   Under these circumstances they were able to isolate 1.6 per cent of racemic serine from silk fibroin.   They also confirmed Cramer's discovery of serine in sericine.   Somewhat later Fischer and Dörpinghaus (178) obtained the same substance from horn, thereby showing that serine is not a unique component of silk proteins.   Kossel and Dakin (183) obtained it from protamines, and within a few years it became clear that serine is a widely distributed amino acid component of proteins.   In 1907 Fischer (177) demonstrated that the serine which results from the hydrolysis of silk is the levorotatory optical isomer and that the inactive products that had hitherto been obtained had been racemized during the process of isolation.

Serine was first synthesized by Fischer and Leuchs in 1902 (180) from glycolic aldehyde by the Strecker cyanohydrin method.   A synthesis by the hippuric acid method has been described by E. Erlenmeyer, Jr. (176) and a synthesis from chloracetal, in which the chlorine atom is replaced by an ethoxy group by means of sodium ethylate, and the resulting acetal is hydrolyzed and employed in a cyanohydrin reaction, has been given by Leuchs and Geiger (184).   The resolution of racemic serine was accomplished by Fischer and Jacobs (179) in 1906 by chemical methods and later by Ehrlich (174) by means of yeast.   Serine was found in human sweat by Embden and Tachau in 1910 (175) and was isolated from an extract from green alfalfa leaves by Vickery in 1925 (186).

Daft and Coghill (173) have recently shown that serine is unstable when heated in strongly alkaline solutions, and is decomposed with the production of ammonia, glycine, alanine, oxalic acid, and lactic acid; pyruvic acid is an intermediate product of the highly complex reaction.   This observation has an important bearing on the indirect methods for the analysis of amino acid mixtures.

GLUTAMIC ACID

$$CH_2—CH(NH_2)—COOH$$
$$|$$
$$CH_2—COOH$$

In 1866 Werther, professor of mineralogy at Königsberg and one of the editors of the Journal für praktische Chemie, reported (192) that Professor Ritthausen had given him, for crystallographic measurement, a specimen of beautifully crystalline material together with the information that the substance had been obtained from the gluten of wheat flour. It was a monobasic nitrogenous acid, "deren Formel nach Analyse der freien Säure, des Baryt- und Kupfersalzes ist $C_{10}H_9NO_8$. Ich nenne sie Glutaminsäure mit Rucksicht auf das Material aus dem sie gewonnen ist."[11] In modern atomic weights this formula is $C_5H_9NO_4$ and is correct.

Ritthausen's paper (190) which soon followed, described the method by which the new acid had been obtained. According to Ritthausen, wheat gluten contains three alcohol-soluble proteins, gliadin, gluten-fibrin and mucedin, which differ in their solubility in alcohol-water mixtures. The later investigations of Osborne showed that gluten-fibrin and mucedin were probably merely fractions of the single alcohol-soluble protein gliadin that is present in wheat. So-called gluten-fibrin, however, was the source of the first glutamic acid. The protein was hydrolyzed with sulfuric acid and the reagent was removed by means of calcium hydroxide. Ritthausen observed that an acid sufficiently strong to decompose calcium carbonate was present. The excess of calcium was therefore precipitated with oxalic acid and the excess of this by means of lead carbonate. The solution was now found to contain a soluble lead salt. The lead was therefore removed as sulfide and the solution was concentrated. Crystals of tyrosine, mixed with another more soluble substance, separated. This was dissolved away from the tyrosine by careful treatment with hot water and, when cooled, this solution deposited gleaming rhombic crystals. When gliadin was treated in the same way a

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[11] Although this quotation is from Werther's paper it is clear from the context that he is quoting the words of a communication from Ritthausen; the "ich" therefore refers, not to Werther, but to Ritthausen. Proof of this is found in Ritthausen's first paper (190) in which the following words occur, ". . . . ist bereits bekannt dass ich durch Kochen des Klebers mit Schwefelsäure eine neue stickstoffhaltige Säure erhalten habe welcher ich den Namen Glutaminsäure gab."

yield of 30 per cent of the new acid was secured.   Salts of barium, copper, and silver were prepared and analyzed; the nitrogen was entirely removed from the new acid by treatment with nitrous acid, and the product, glutanic acid, was recognized as being allied to malic acid.   In subsequent publications Ritthausen (191) showed that glutamic acid is present in many other vegetable proteins.

Hlasiwetz and Habermann in 1873 (188) introduced the method of hydrolyzing proteins with hydrochloric acid in the presence of stannous chloride, added to prevent the formation of humin, and showed that glutamic acid can be conveniently isolated as its hydrochloride.   They prepared this from casein, the first protein of animal origin shown to yield glutamic acid.   As a result of their experiments they drew the deduction that casein yields exclusively leucine, tyrosine, glutamic and aspartic acids, and ammonia, and pointed out that the ammonia, which is an invariable product of protein hydrolysis, probably "von jener, im Casein primär enthaltenen Verbindungen abstammt, welche gleichzeitig Asparaginsäure und Glutaminsäure liefern."   They suggested that a close analogy existed between the ammonia derived from proteins and that derived from asparagine and glutamine.   "Verbindungen dieser Art welche beim Erhitzen mit Säuren oder Alkalien unter Wasseraufnahme Ammoniak verlieren und diese Säuren liefern, müssen im Casein und den Proteinstoffen überhaupt präexistirend angenommen werden."

The product of the action of nitrous acid on glutamic acid, described by Ritthausen as glutanic acid, was reduced with hydriodic acid by Dittmar (186) to a substance that Markownikoff (189) showed to be identical with the product of hydrolysis of trimethylene cyanide $CNCH_2$—CH_2—CH_2CN.   Glutanic acid was therefore an hydroxyglutaric acid and, since it was different from the already known β-hydroxyglutaric acid, it could only be α-hydroxyglutaric acid.   Glutamic acid was therefore α-aminoglutaric acid, $HOOC$—CH_2—CH_2—$CH(NH_2)COOH$.   Final proof was secured by Wolff (193) who synthesized glutamic acid from levulinic acid by a most unusual method.   Levulinic acid was brominated yielding a product which, when boiled with

water, yielded glyoxylpropionic acid. This was treated with hydroxylamine, and the product was converted to a furazane derivative by sulfuric acid. This, in turn, was converted to cyanonitrosobutyric acid by sodium hydroxide hydrolysis; further hydrolysis and reduction of this gave glutamic acid.

$$
\begin{array}{ccccc}
\text{CH}_3 & \text{CH}_3 & \text{CHO} & \text{CH}{=}\text{NOH} \\
| & | & | & | \\
\text{CO} & \text{CO} & \text{CO} & \text{C}{=}\text{NOH} \\
| & | & | & | \\
\text{CH}_2 \longrightarrow & \text{CBr}_2 \longrightarrow & \text{CH}_2 \longrightarrow & \text{CH}_2 \longrightarrow \\
| & | & | & | \\
\text{CH}_2 & \text{CH}_2 & \text{CH}_2 & \text{CH}_2 \\
| & | & | & | \\
\text{COOH} & \text{COOH} & \text{COOH} & \text{COOH}
\end{array}
$$

Levulinic   Dibromolevulinic   Glyoxyl-   Diisonitroso
acid   acid   propionic   valeric acid
        acid

$$
\begin{array}{cccc}
\text{CH}{=}\text{N} & \text{CN} & \text{COOH} & \text{COOH} \\
| \quad {>}\text{O} & | & | & | \\
\text{C}{=}\text{N} & \text{C}{=}\text{NOH} & \text{C}{=}\text{NOH} & \text{CHNH}_2 \\
| & | & | & | \\
\text{CH}_2 \longrightarrow & \text{CH}_2 \longrightarrow & \text{CH}_2 \longrightarrow & \text{CH}_2 \\
& & & \\
\text{CH}_2 & \text{CH}_2 & \text{CH}_2 & \text{CH}_3 \\
| & | & | & | \\
\text{COOH} & \text{COOH} & \text{COOH} & \text{COOH}
\end{array}
$$

         Cyanonitrosobutyric acid   Glutamic acid

Curiously enough there appears to be no other recorded synthesis of glutamic acid.

It is noteworthy that glutamic acid appears to be the only amino acid which has found commercial use. The monosodium salt is widely used in the Orient as a condiment (187).

PHENYLALANINE

$$\langle\bigcirc\rangle\text{CH}_2\text{—CH(NH}_2)\text{—COOH}$$

If the synthesis of phenylaminopropionic acid from bromohydrocinnamic acid and ammonia by Posen (205) in 1879 can be accepted without question, the discovery of phenylalanine must

be simultaneously credited to both Posen and to Schulze and Barbieri (210). Posen obtained a product which, on analysis, yielded figures in agreement with those to be expected from phenylaminopropionic acid. It was slightly soluble in cold water, more so in hot water, and its behavior towards acids and alkalies showed that it possessed amphoteric properties. He stated, however, that it was soluble in alcohol and melted at 120–121°, which suggests benzoic acid rather than phenylalanine. It is quite possible that Posen by mistake carried out his melting point estimation on benzoic acid and not phenylalanine. On account of these discrepancies it is difficult to accept Posen's work although his claim cannot be wholly rejected.

A preliminary note, by Schulze and Barbieri, of the discovery of a new amino acid in the etiolated sprouts of *Lupinus luteus* was published in 1879. They extracted the sprouts with alcohol, added lead acetate, filtered off the precipitate, freed the filtrate from lead and concentrated it. A small amount of leucine, traces of tyrosine, and a considerable amount of asparagine and the new amino acid were obtained. This was partially purified by means of the copper salt. Analysis showed that a substance was present that contained about 10 per cent more carbon than does leucine, but they made no statement as to the possible structure of the new substance.

Two years later Schulze and Barbieri (211) reported the results of more extended investigations of lupine seedlings. An extract was prepared as before, the crystalline mass secured by evaporation was treated with alcohol that contained a little ammonia; asparagine remained undissolved and the alcoholic solution yielded crystals on evaporation. These were recrystallized several times, a crystalline copper salt was prepared, decomposed with hydrogen sulfide and the free acid was again secured. This material was taken through the copper salt stage a second time; the final product then crystallized in plates or leaves, or from dilute solution in hydrated needles. The analysis led to the correct formula $C_9H_{11}NO_2$ and oxidation yielded benzoic acid. Dry distillation gave a product that appeared to be phenylethylamine. "Unsere Amidosäure ist demnach als eine Phenylamidopropion-

säure anzusehen. . . . ." Tiemann (216) had already pointed out the possible existence of aromatic amino acids in the native protein molecule. "Die Bildung von Benzöesäure und Benzaldehyd bei der Oxydation der Eiweisskörper . . . . deuten darauf hin, dass in den Proteinsubstanzen auch Reste von Monosubstitutionsproducten des Benzols vorkommen."

There are four possible isomeric phenylamidopropionic acids which can be represented by the composition of the new substance. The product secured by Schulze and Barbieri melted at 250° which, in view of Posen's findings, excluded the possibility that it might be α-aminophenylpropionic acid. Similarly the possibility that it might be aminohydratropic acid, which melted at 169°, was also excluded. Schulze and Barbieri were inclined to favor the view that their amino acid was a homologue of phenylaminoacetic acid but made no definite commitment at this time.

Schulze and Barbieri observed that, like asparagine, little or no phenylalanine is found in the free state in the seeds of *Lupinus luteus* before germination. "Denn wir wissen, dass während der bei Lichtabschluss stattfindenden Keimung ein beträchtlicher Theil von den Eiweissstoffen der Samen zersetzt wird; das Asparagin, welches in Lupinenkeimlingen in so bedeutender Quantität auftritt, kann nur aus Eiweissstoffen entstanden sein; denn nicht eiweissartige Stickstoffverbindungen finden sich in den ungekeimten Lupinensamen nur in so geringer Menge vor, dass sie nicht entfernt hinreichen, um die im Asparagin sich vorfindende Stickstoffmenge zu liefern; die Annahme, dass auch die neben Asparagin sich vorfindenden Amidosäuren dem Zerfall von Eiweiss entstammen, dürfte also wohl eine sehr wahrscheinliche sein."

The new acid was therefore sought among the products of hydrolysis of lupine seed proteins. Extensive fractional crystallization did, indeed, give fractions that were mixtures of leucine with a substance that yielded benzoic acid on oxidation and the previously found volatile base on dry distillation; there was little doubt, therefore, that the phenylaminopropionic acid originated from the protein of the seed.

Less definitely characterized than the products of Posen and

of Schulze and Barbieri was the tyroleucine obtained by Schützenberger (214) in 1879. He regarded tyroleucine as being a combination of aminovaleric acid and a substance of the formula $C_9H_{11}NO_2$, which is that of phenylalanine. The indefiniteness of Schützenberger's work, however, lays it open to question. Schulze (208) believed that Schützenberger's leucëines may very possibly have included phenylalanine.

A year after the appearance of Schulze and Barbieri's paper, Erlenmeyer and Lipp (198) reported the synthesis of phenyl-α-aminopropionic acid, a term which they shortened to phenylalanine. Phenylacetaldehyde was treated with hydrocyanic acid and ammonia in accordance with the Strecker method and the nitrile was saponified to give phenylalanine.

Schulze and Barbieri (212) were quick to see the possible relationship of the amino acid they had isolated from *Lupinus luteus* to the synthetic product of Erlenmeyer and Lipp. They concluded ". . . . dass sie höchst wahrscheinlich identisch mit der von E. Erlenmeyer und A. Lipp vor kurzem synthetisch dargestellten Phenyl-α-Amido-propionsäure (Phenylalanin) ist. . . . ." In a subsequent publication Schulze and Barbieri (213) were able to isolate phenylalanine from the proteins of squash seed after acid hydrolysis as well as after hydrolysis with barium hydroxide (208). This definitely proved that phenylalanine is a product of the hydrolysis of the protein molecule. They also showed that, on heating phenylalanine, phenyl-lactimide and phenylethylamine were formed. The latter substance was the same as that which Erlenmeyer had found on heating his synthetic phenylalanine. This established the structural identity of the synthetic phenylalanine and the phenylalanine isolated from natural sources. Schulze (209) also showed that phenylalanine together with asparagine, glutamine, leucine, valine, tyrosine, guanidine, choline, and betaine, were present in etiolated vetch sprouts.

The subsequent work on phenylalanine has been chiefly concerned with its synthesis. No other amino acid has received as much attention from this standpoint and no other amino acid has been synthesized in so many different ways as has phenyl-

alanine. For this reason, the various synthetic reactions are given in detail. Erlenmeyer, Jr., (196) converted phenylpyruvic acid into the oxime which, on reduction, yielded phenylalanine. Shortly afterwards, he published a second method (197); benzaldehyde was condensed with hippuric acid in the presence of acetic anhydride. The lactimide was converted into benzoylaminocinnamic acid by hydrolysis. Reduction and subsequent hydrolysis of this yielded phenylalanine. Knoop and Hoessli (204) confirmed this synthesis. They used aluminum amalgam for the reduction. Fischer's (199) method consists in treating benzylmalonic ester with bromine, converting the resultant benzylbromomalonic acid into phenyl-α-bromopropionic acid by heating, and finally treating with ammonia. By treating cinnamic acid with hydroxylamine, Posner (206) obtained α-oxyamino-β-phenylpropionic acid which, on reduction with ammoniacal silver solution, gave phenylalanine. Sörensen (215) used his phthalimide method for the synthesis of phenylalanine. Bromomalonic ester is treated with potassium phthalimide to form phthalimidomalonic ester which is converted, by treatment with sodium alcoholate, into the sodium salt. This compound is converted, by benzyl chloride, into benzylphthalimidomalonic ester; saponification of the ester with sodium hydroxide yields the sodium salt of phthalimidobenzylmalonic acid which, by the action of hydrochloric acid, is split into phenylalanine, phthalic acid and carbon dioxide. Wheeler and Hoffman's (217) synthesis consists in condensing benzaldehyde with hydantoin to form benzalhydantoin; reduction with hydriodic acid and red phosphorus yields the hydantoin of phenylalanine and hydrolysis with barium hydroxide gives phenylalanine.

Wheeler and Hoffman's method was improved by Johnson and O'Brien (203). They treated hippuric acid with potassium thiocyanate to form 2-thio-3-benzoylhydantoin; on condensation with benzaldehyde, 3-benzoyl-2-thio-4-benzalhydantoin was obtained. This compound was hydrolyzed with warm hydrochloric acid which yielded benzalthiohydantoin; on reduction with tin and hydrochloric acid, the ring was broken and phenylalanine obtained.

Sasaki (207) has proposed a method which can be used for the synthesis of both phenylalanine and tyrosine. The yield is about 83 per cent. Glycine anhydride and benzaldehyde are condensed in the presence of acetic anhydride and sodium acetate to give 3,6-dibenzal-2,5-diketopiperazine. On reduction with hydriodic acid and red phosphorus, the diketopiperazine ring is split and phenylalanine is obtained. The procedure of Curtius and Sieber (194) is somewhat longer. The potassium salt of benzylmalonic acid is treated with hydrazine to form the hydrazide of benzylmalonic acid. The latter substance is treated with sodium nitrite in the cold; this forms the azide of benzylmalonic acid. On heating the latter compound, nitrogen is split off and the anhydride of phenylalanine-N-carbonic acid is formed. On heating with hydrochloric acid, hydrolysis with simultaneous loss of carbon dioxide takes place, yielding phenylalanine. Harington and McCartney (202) have recently improved the method of Erlenmeyer, Jr. (197).

Fischer and Mouneyrat (200) were the first to resolve racemic phenylalanine into its optically active components. This was accomplished by means of the cinchonine salts of the benzoyl derivatives. They showed that the magnitude of the specific rotation of d-phenylalanine to the right-was essentially the same as Schulze and his co-workers (213) found for the naturally occurring phenylalanine which rotates the plane of polarized light to the left. Later Fischer and Schoeller (201) carried out the resolution by means of the brucine salts of the formyl derivatives. They were also able to convert the ethyl ester of l-phenylalanine into the ethyl ester of d-α-bromohydrocinnamic acid. This was one of the first examples of the Walden inversion in the field of amino acids. Ehrlich (195) was able to resolve racemic phenylalanine with the aid of yeast, which does not destroy the dextro form. Fischer (199) and Fischer and Schoeller (201) were the first to prepare a series of peptides of phenylalanine and glycine.

The isolation from proteins of a second amino acid that contained an aromatic nucleus was a matter of the greatest importance and the interest that was aroused by the discovery of phenylalanine is reflected in the extent of the work on the synthe-

sis of this substance.   It may also be mentioned that, previous to 1901, synthesis was almost the only sure way in which a specimen of phenylalanine could be secured.   Schulze's isolation by direct crystallization methods furnishes an impressive example of the great technical skill of this investigator and it is doubtful if pure specimens of this substance were again isolated from natural sources until Fischer developed the ester distillation method of protein analysis.   It was Fischer who demonstrated that phenylalanine, far from being an extremely rare substance, is widely distributed in nature.

LYSINE

$$CH_2—CH(NH_2)—COOH$$
$$|$$
$$CH_2—CH_2—CH_2—NH_2$$

Hlasiwetz and Habermann (227) in 1873 showed that proteins could be advantageously hydrolyzed by means of hydrochloric acid in the presence of tin.   They believed that the decomposition took place smoothly and that the products were leucine, tyrosine, glutamic and aspartic acids and ammonia, with only a small residue of unknown material.   Schützenberger (231) in 1879 decomposed proteins by heating them at 150° with barium hydroxide and likewise obtained aspartic and glutamic acids, tyrosine, and leucine, and in addition, a series of substances that he regarded as homologues of leucine.   Other substances were found in small amounts but these seemed to be the chief products. Schulze (230) in 1885 pointed out the improbability that only four substances resulted from the hydrolysis of proteins and showed that the newly discovered phenylalanine was likewise a product.

The whole problem of the completeness with which a protein could be accounted for in terms of its products of hydrolysis was discussed by Drechsel (219) in 1889.   Drechsel was particularly impressed by some work of Horbaczewski (228) who had accounted for only about 30 per cent of the keratins of horn as definite substances.   Furthermore he was intrigued by the carbon dioxide observed by Schützenberger when proteins were heated

with alkali. He satisfied himself that little, if any, carbon dioxide was produced by acid hydrolysis and drew the conclusion, "dass bei der Spaltung der Eiweisskörper durch concentrierte Salzsäure noch andere, bisher noch nicht aufgefundene Produkte entstehen, welche sich in den Mutterlaugen der genannten Amidokörper finden, und wenigstens zum Theil beim Erhitzen mit Barythydrat Kohlensäure liefern müssen." Drechsel therefore repeated the experiment of Hlasiwetz and Habermann; he hydrolyzed casein by long-continued boiling with hydrochloric acid in the presence of stannous chloride, removed the tin, crystallized out as much crude glutaminic acid hydrochloride as possible and then diluted the sirupy mother liquor and treated it with phosphotungstic acid, a reagent long employed for the precipitation of alkaloids and recently used with remarkable success (see arginine) by Schulze in the investigation of plant extracts. The heavy precipitate was removed, washed, and decomposed with barium hydroxide, the excess of this reagent was carefully precipitated and the solution was acidified with hydrochloric acid and concentrated to a sirup; on standing a crystalline substance separated. This was redissolved, the solution was diluted with alcohol, and ether was added. The oil which separated crystallized in part on standing and the crystals were removed, pressed out dry on a porous tile and washed with absolute alcohol. The substance was recognized as the hydrochloride of a strong base since its solution, on treatment with silver carbonate to remove the chloride, was strongly alkaline. The chloroplatinate was therefore prepared, and was found to crystallize from dilute alcohol in long prisms. Analysis of this led to the formula $C_7H_{14}N_2O_2PtCl_6 + 4H_2O$. The mother liquors of the chloride yielded a chloroplatinate different in color from the first and of a composition that agreed with the formula $C_8H_{16}N_2O_2Cl_2 \cdot PtCl_4 + H_2O$. This substance was thought to be homologous with the first base. It is to be noted, however, that Drechsel did considerable violence to the analytical figures in ascribing this formula to it. The most important property of these substances that was recorded in the preliminary paper was the decomposition by means of alkali. The crude chloride was stable when heated

to 150° with acids but, when heated with barium hydroxide to 120°, decomposition took place with the liberation of barium carbonate. This behavior gave a clue to the observations of Schützenberger: "diese Basen sind die oder eine Quelle der Kohlensäure, welche Schützenberger fand."

Drechsel in the following year continued his study of the basic substance derived from proteins (220). In addition to the substance that formed a crystalline chloroplatinate a second base was isolated, as an acid double salt of silver nitrate, of the composition $C_6H_{13}N_3O_2 \cdot HNO_3AgNO_3$. He thought that the base contained one molecule of water of crystallization; it should therefore have the composition $C_6H_{11}N_3O$ which suggested that the new substance was homologous with creatine $C_4H_9N_3O_2$ and creatinine $C_4H_7N_3O$ "und die Vermuthung lag deshalb nahe, dass diese Basen, welche Lysatin bezw. Lysatinin heissen mögen, auch wirklich die Constitution des Kreatins bez. Kreatinins besitzen möchten." As a demonstration that this view was correct, Drechsel subjected the new substance to a short hydrolysis with barium hydroxide and then isolated urea from the products of the decomposition. Although urea and guanidine had previously been obtained from proteins in small amounts by oxidation, this was the first time that urea had been obtained from a protein by purely hydrolytic processes. The name lysatine (λύσις, loosing), applied by Drechsel to his new substance, illustrates the importance he attached to this fact.

The meaning of these preliminary observations did not become clear for some time. At least two different basic substances were present and one of these had been isolated in pure form, but the properties of the other substance were so confusing that several years elapsed before a full explanation was forthcoming. As will appear, the development of the chemistry of the basic amino acids depended entirely on the selection of the right reagent to employ at each stage. Drechsel made an immense advance when he first thought of applying phosphotungstic acid to the investigation of the products of protein hydrolysis, but the separation of the mixture of bases precipitated by this reagent required years of study.

The next publication from Drechsel's laboratory appeared early in 1891 (232).   Drechsel had suggested to Siegfried that he should investigate other proteins than casein to see if the new basic substances were to be found in them.   Conglutin, gluten-fibrin (probably gliadin), "hemiprotein," "oxyprotsulfonic acid," and egg albumin were therefore studied.   These were commercial preparations probably secured from the firm of Grübler.   In the course of the investigation of conglutin Siegfried made an ex-tremely important observation which was later, in the hands of Kossel, to serve as the key to the whole problem of separating the basic amino acids.   He said, "Ohne vorherige Fällung mit Phosphorwolframsäure würde man durch Silbernitrat aus den Zersetzungsproducten der Eiweisskörper durch Salzsäure nicht lediglich Asparaginsäure fällen, da, wie ich weiter unten zeigen werde, sich stets eine durch Phosphorwolframsäure fällbare Base vorfindet, welche ebenfalls ein in Wasser unlösliches amorphes Silbersalz bildet."   He did not, however, work on this amorphous silver compound but studied the substances precipitated by phosphotungstic acid.   His use of silver nitrate was, however, a direct outcome of Drechsel's observation that the base lysatine formed a crystalline double salt with this reagent; Drechsel was therefore the first to employ silver as a reagent in this field.

Siegfried isolated the base that formed a crystalline chloroplat-inate and showed that it had the composition $C_8H_{22}N_2O_8Cl_6Pt$. When this salt was decomposed with hydrogen sulfide and the resulting hydrochloride was boiled with barium hydroxide, neither carbonate, acetic acid, oxalic acid, nor alcohol was pro-duced; the preparation was therefore free from lysatine.   After removal of the barium, the hydrochloride was obtained in crystals of the composition $C_6H_{14}N_2O_2 \cdot 2HCl$ and this prepara-tion, treated with chloroplatinic acid yielded a substance identical with the initial chloroplatinate.   It was obvious therefore that the two extra carbon atoms in this salt represented alcohol of crystallization and that its composition should be expressed $C_6H_{14}N_2O_2 \cdot C_2H_5OH \cdot H_2PtCl_6$.   That this was the case was shown by a demonstration of alcohol in the crystalline salt. Siegfried secured specimens of the same salt from each of the

materials he investigated and it became clear that the new base was widely distributed in nature.

Drechsel and his assistants continued the investigation in a long paper (221), the greater part of which appeared under Drechsel's name, but sections of which were furnished by Siegfried, by Ernst Fischer, and by S. G. Hedin. A large quantity (10 kilograms) of casein was hydrolyzed and the basic substances were precipitated by phosphotungstic acid. The volatile base in the precipitate was shown to be ammonia, and the solution of the fixed bases was neutralized with hydrochloric acid. From this an impure chloride was obtained which did not give a satisfactory analysis; it was therefore recrystallized from concentrated hydrochloric acid, whereby a dichloride was obtained that analyzed sharply for a diaminocaproic acid. The monochloride was found to be only slightly acid to litmus. The formula $C_6H_{14}N_2O_2$ was deduced for the free base, and Drechsel pointed out that this was homologous with ornithine and that the substance itself had certain properties in common with ornithine. The name lysine is first employed as a designation for this base in the section of the paper by Ernst Fischer. He refers in the last paragraph to "die Base $C_6H_{14}N_2O_2$, welche mit dem Namen Lysin bezeichnet werden mag," and goes on to point out that it can be obtained by alkaline hydrolysis of gelatin under pressure and must therefore have been present in the solutions investigated by Schützenberger.

The last section of the paper was written by S. G. Hedin and describes the isolation of lysine chloroplatinate from a pancreatic digest of fibrin. Hedin showed that some of the same substance was formed by autolysis of the pancreas powder he employed, but that the much larger quantity isolated from the digest established the fact that lysine is a product of the digestion of fibrin. He could not, however, obtain more than traces of the second base, lysatine.

A final paper on lysine from Drechsel (222) recorded his attempt to crystallize the free base; the solid material secured was, however, a carbonate. Crystalline lysine was first prepared by Vickery and Leavenworth (234) in 1928. Drechsel's paper con-

tains the suggestion that lysine is a derivative of pentamethylene-diamine; this was demonstrated to be the case, in 1899, when Ellinger (223, 224) showed that pentamethylenediamine could be obtained from lysine by anaerobic putrefaction. He suggested that the most probable constitution was expressed by the formula

$$NH_2CH_2-CH_2-CH_2-CH_2-CH(NH_2)-COOH$$

although the position of the carboxyl was not certainly established. Henderson (226) pointed out that the carboxyl could not be on the middle carbon atom because of the optical activity, and his demonstration that acetic and propionic acids were formed by alkali fusion strongly supported the view that the carboxyl was at the end of a straight chain. The synthesis of lysine by Fischer and Weigert (225) from γ-cyanopropylmalonic ester by treatment with nitrous acid followed by reduction finally settled the matter. Other syntheses have since been described by Sörensen (233) and by von Braun (218).

The importance of lysine in animal nutrition was first demonstrated in 1914 by Osborne and Mendel (229), who found that this amino acid behaves as a limiting factor on growth. Animals did not grow when the lysine-deficient gliadin formed the chief protein of the diet. When small doses of lysine were added to the diet, however, normal growth occurred. Without this addition animals could be maintained for long periods at approximately constant weight.

<div align="center">ARGININE</div>

$$CH_2-CH(NH_2)-COOH$$
$$|$$
$$CH_2-CH_2-NH-C(NH_2)=NH$$

In 1886 Schulze and Steiger announced their discovery of a new basic substance in extracts from etiolated lupine seedlings in a paper (244) that is a model of what such papers should be; concise and clear, each statement supported by exact experimental work and competent analysis that leaves no doubt as to the accuracy of the findings.

Schulze had long known that phosphotungstic acid gave white precipitates when added to plant extracts and also that these precipitates contained nitrogen. Among the substances in them he had recognized what he called peptones, since they gave a precipitate with tannic acid and also responded to the biuret test. These early observations had not been followed up owing to the pressure of other problems, and also because Schulze had felt that it would be unusually difficult to isolate definite substances from these precipitates.

Schulze and Steiger prepared a water extract of etiolated lupine seedlings, treated this successively with tannic acid and with lead acetate, removed the precipitates and, after acidifying with sulfuric acid and removing the lead sulfate, added an excess of phosphotungstic acid. The precipitate was decomposed with calcium hydroxide and the excess of this was removed as carbonate. The alkaline solution was neutralized with nitric acid and when evaporated to a thin sirup, "so krystallisirt aus derselben das salpetersaure Salz einer Base, welcher wir den Namen Arginin beilegen wollen." The nitrate had the composition $C_6H_{14}N_4O_2 \cdot HNO_3 \cdot 1/2H_2O$ and this formula was supported by analyses of the hydrochloride and of the insoluble copper nitrate double salt. The new base could be precipitated at neutral or alkaline reaction by mercury salts and the addition of sodium carbonate was especially advantageous. The yield of arginine obtained amounted to between 3 and 4 per cent of dry weight of the cotyledons. The substance could not be identified with any previously known but, in some points, it resembled creatinine.

A second paper (245), in which a description of a number of other compounds of arginine was given, soon followed. The new base was stable when heated with acids—even hydriodic acid did not affect it—but it was slowly decomposed by alkalies with the formation of carbon dioxide and ammonia and of a strongly basic substance which yielded a crystalline chloride and sulfate.[12]

Arginine contained about one-third of its nitrogen in a form

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[12] This substance was identified in a later paper (246) as ornithine by means of its dibenzoyl compound, ornithuric acid, discovered many years before by Jaffé (239).

readily removed by nitrous acid; it contained neither sulfur nor phosphorus; it yielded precipitates with most of the so-called alkaloid reagents; it was present in the sprouts of squash seeds. Schulze was of the opinion that the new base arose from the decomposition of the protein of the seed during germination, and Drechsel's discovery of basic substances among the products of acid hydrolysis of proteins caused him to return to the study. The amount of arginine produced during the sprouting of lupine seeds was shown to exceed (242) the equivalent of the non-protein nitrogen of the seed. Protein must therefore have been converted, in part at least, into arginine during the germination process. This observation at once associated the new basic substance with proteins.

The next step was the demonstration that arginine could be decomposed by alkali with the production of urea. Schulze and Likiernik (243) pointed out that Drechsel's discovery of the two new bases, lysine and lysatine, among the products of hydrolysis of proteins greatly increased the significance of the relationships of basic substances to the chemistry of natural processes, and their observation that arginine, like lysatine, yielded urea when heated with alkali was a matter of the greatest importance. "Da Lysatin und Lysin in den argininhaltigen Keimpflanzen nicht enthalten zu sein scheinen, so darf es wohl für wahrscheinlich gelten, dass diejenigen Atomgruppen im Eiweissmolekül welche beim Kochen der Eiweissstoffe mit Salzsäure die eben genannten Basen liefern, bei dem im Keimpflanzen erfolgenden Eiweisszerfall zur Bildung des Arginins verwendet werden." Thus, as early as 1891, Schulze associated arginine with Drechsel's new base.

The discovery of new amino acids has again and again been a matter of learning to use a reagent in a new way; the isolation of arginine from proteins is an excellent example of this. Siegfried's paper (249) on the isolation of lysine from conglutin, which appeared just before the above mentioned papers of Schulze, contains a number of references to the behavior of protein hydrolysates, and the basic fractions therefrom, when silver nitrate is added. It will be recalled that lysatine was isolated by Drechsel

and by Siegfried as an acid silver nitrate double salt. It will be interesting to see how narrowly, in 1891, the Leipsic group missed adding the discovery of arginine and histidine to their brilliant discovery of lysine. Arginine forms an amorphous silver compound that is soluble in acid but is entirely insoluble in strongly alkaline solutions. Siegfried added silver nitrate to the solution of the free bases obtained from protein hydrolysates by precipitation with phosphotungstic acid; this solution was, of course, strongly alkaline. A voluminous precipitate formed and the reagent was added until no further material separated; the precipitate was removed and was found to contain no nitric acid. Nitric acid equivalent to the silver in the precipitate had therefore been added to the filtrate. The filtrate was then evaporated to a sirup; on standing a sticky, partially crystalline precipitate, which contained lysine, separated. The filtrate was then treated with alcohol when the *acid* silver nitrate double salt of lysatine, $C_6H_{13}N_3O_2 \cdot HNO_3AgNO_3$ began to separate. "Sobald sich einige dieser Krystalle bilden, giesst man die Lösung ab und spült das Gefäss und den am Boden festsitzenden schmierigen Niederschlag mit absolutem Alkohol ab. Durch Zusatz von Aether bewirkt man das vollständige Auskrystallisiren des Silberdoppelsalzes." This technique was hardly calculated to produce a pure substance yet the material was regarded, after a second precipitation in the same way, as pure lysatine.

The amorphous silver precipitate that separated first was apparently neglected; Siegfried carried out some analyses and obtained figures that suggested the formula $C_{11}H_{17}N_6O_6Ag_3$. A quantity of it secured from egg albumin was decomposed with hydrochloric acid and the resulting solution, after evaporation and treatment with alcohol and ether, yielded prismatic crystals that analyzed for $C_{11}H_{20}N_6O_6 \cdot 2HCl$. As will appear later this was almost certainly impure histidine, while the solution from which he separated his lysatine would have yielded crystalline arginine silver nitrate if he had hit upon the correct way to treat it.

Another member of the Leipsic group, Ernst Fischer (235), had prepared lysatine from gelatin, had heated the product with baryta until the urea that it produced was decomposed, and had

then isolated lysine from the resulting solution as a crystalline chloroplatinate. Unfortunately, however, instead of crystalline lysatine he had used a crude mixture for this experiment, and the logical conclusion that lysatine either contained, or yielded, lysine escaped him.

The third junior member of the group, S. G. Hedin, was familiar with these experiments. He had been assigned the problem of isolating lysine and lysatine from tryptic digests of fibrin. He had succeeded in preparing lysine but had failed to obtain lysatine. His preparation, after three crystallizations from alcohol and ether, separated in white needles, the appearance, behavior, and manner of preparation of which appeared to indicate that the compound must be the silver nitrate double salt of lysatine. "Indessen gaben die Analysen keine mit den von der Formel verlangten völlig übereinstimmenden Werthe. Zur weitere Reinigung des Salzes fehlte mir vorläufig an Material, doch gedenke ich die Versuche baldmöglichst wieder aufzunehmen und diesen Punkt klarzustellen."

This failure evidently disturbed him and, after returning to Lund, he repeated the work. In June 1894 he presented a paper (236) on a hydrolysate of horn in which he showed that a compound of the composition of lysatine silver nitrate could be obtained. It separated as crystals mixed with considerable oily material. The oil was separated, freed from silver and treated with chloroplatinic acid. Nothing but oil could be brought to separate. The new oil was removed, freed from platinum and the solution of the chloride, after evaporation to a sirup, was treated with alcohol and ether. The oily precipitate was removed and again treated with chloroplatinic acid. This time it crystallized and the product was immediately recognized as lysine chloroplatinate. Crude lysatine therefore contained lysine.

This gave Hedin an idea. He repeated the work on a larger scale and added silver nitrate to the base fraction as before, removed the amorphous silver precipitate and concentrated the solution to a thin sirup, *but did not add alcohol*, evidently feeling that alcohol precipitated too much. A crystalline substance slowly separated which was recrystallized from water and an-

alyzed. It gave figures for $C_6H_{14}N_4O_2 \cdot AgNO_3 \cdot 1/2H_2O$. This was new; no compound hitherto secured from proteins contained as many as four nitrogen atoms to six carbon atoms. He carried the salt back through the phosphotungstate to free base, prepared the same silver salt from this again, and also observed that the mother liquor contained a more soluble salt that crystallized in needles and had the composition $C_6H_{14}N_4O_2 \cdot AgNO_3 \cdot HNO_3$. On removal of silver, the nitrate $C_6H_{14}N_4O_2HNO_3 \cdot 1/2 H_2O$ was obtained. From this a double salt with copper nitrate that crystallized with three molecules of water of constitution was secured. This clinched the identity; the substance was Schulze's arginine. Analyses and properties of copper salt and nitrate were identical.

The following year Hedin reported (237) the results of a vast amount of work. He showed that arginine formed two salts with nitric acid, a mono- and a di-nitrate; he prepared the mono-chloride but could not make the dichloride crystallize. A specimen of arginine, secured from Schulze, was shown to yield these salts as well as his two new silver double salts. Furthermore, he demonstrated that arginine from protein yielded carbon dioxide when heated with barium hydroxide, although he did not succeed in isolating enough urea from the reaction mixture for positive identification through analysis.

He showed that when silver nitrate is added to the alkaline solution of the bases, inasmuch as this always contained carbonate, silver carbonate was precipitated, thereby liberating nitric acid. Furthermore, the amorphous silver precipitate contained "andere anwesenden, nicht näher bekannten Basen als Silberverbindungen," but no nitric acid; the removal of silver by the precipitation of this material thereby set free still more nitric acid. Consequently the crystallization of the double silver salt of arginine could only proceed incompletely. By neutralization of the liberated nitric acid, however, a much more complete crystallization could be secured. Hedin investigated a number of proteins and found arginine in them all. In each case he observed that the mother liquor of the insoluble silver double salt contained the silver salt of another base.

A few months later his investigation of this was complete and

an explanation of the whole lysatine problem was reached (238). The solution of the bases derived from casein was treated with silver nitrate, the amorphous silver precipitate was removed and the filtrate was treated with barium hydroxide until brown silver oxide began to precipitate. Carbon dioxide was then passed in, the precipitate was removed and the filtrate was concentrated. Barium nitrate crystallized first from this alkaline solution and was followed by the silver nitrate double salt of arginine in small amount. Further evaporation brought about the separation of a mass of white crystals which, however, could not be satisfactorily purified by recrystallization. The addition of alcohol did not help. Hedin had observed that arginine forms two compounds with silver nitrate, a relatively insoluble double salt that crystallized with one-half molecule of water from somewhat alkaline solutions, and a much more soluble acid salt that crystallized with one molecule of nitric acid. The mother liquors of the arginine silver nitrate obviously contained another base which forms a silver nitrate double salt. In order to see if it also formed an acid silver nitrate double salt he added enough nitric acid to give a weakly acid reaction and then added alcohol. A crystalline substance immediately separated which, after recrystallization from dilute alcohol with the aid of ether, turned out to be the acid silver nitrate double salt of lysine. The same salt was readily prepared from lysine chloroplatinate. This cleared up the whole situation. Both arginine and lysine form two silver salts. The silver nitrate double salt of arginine is relatively insoluble and is easily prepared. The acid silver nitrate double salt of arginine is much more soluble. The acid silver nitrate double salt of lysine is a decidedly soluble salt but its neutral, or rather alkaline, silver nitrate double salt is very soluble. The pairs of compounds are analogous but the solubilities are in reversed order. When both bases are present in a solution together with silver nitrate, and the solution is treated with alcohol and ether as had been the custom, a mixture of the two less soluble salts separated, namely arginine silver nitrate and acid lysine silver nitrate. The product that had been obtained by Drechsel, Siegfried, Fischer, and himself and

recognized as lysatine silver nitrate had a composition almost exactly half-way between that of the arginine and lysine silver nitrates. "Wohl geht es aus meinen Versuchen nicht unzweideutig hervor, dass das Lysatinin als chemisches Individuum nicht existirt, aber so viel scheint doch bewiesen zu sein, dass das Lysatininsalz, nach üblichen Methoden dargestellt, beträchtliche Mengen Arginin und Lysin enthalten muss." Thus Hedin gently buried the mistake of his distinguished teacher.

Arginine rapidly assumed importance in protein chemistry. Kossel demonstrated (240) that it was unusually plentiful in the basic protein-like substances he had prepared from fish sperm. In 1897 Schulze and Winterstein (246, 247) found that arginine, when heated with alkalies, yielded ornithine in addition to urea. This was isolated as its dibenzoyl compound, the ornithuric acid (ὄρνιθος, bird; urina, urine) of Jaffé (239). They therefore suggested that arginine probably had the constitution,

$$NH_2 \qquad\qquad\qquad NH_2$$
$$| \qquad\qquad\qquad\qquad |$$
$$NH{=}C{-}NH{-}CH_2{-}CH_2{-}CH_2{-}CH{-}COOH$$

and that a substance of this constitution could probably be obtained by the action of cyanamide on ornithine. That this was the case was shown in 1899 (248), although the position of the guanidino group was not established by this synthesis. It was merely assumed that this group was attached to the nitrogen atom in the δ-position.

Definite proof of the structure of arginine was secured only in 1910. Sörensen (250) found that ornithuric acid, when hydrolyzed by acid, gave δ-benzoylornithine, but when hydrolyzed by alkalies it gave α-benzoylornithine. The condensation of cyanamide with these isomeric substances, followed by hydrolysis of the benzoyl group with strong acid, gave two isomeric basic substances of which the product from α-benzoylornithine was identical with racemic arginine.

The story of arginine would be incomplete without reference to Kossel's return to the subject a few years before his death. In 1924 Kossel and Gross (241) made the brilliant observation that

arginine forms a highly insoluble and beautifully crystalline compound with 2,4-dinitro-α-naphthol-7-sulfonic acid, or flavianic acid, as they suggested that this compound might more conveniently be called. This observation has led to a complete revision of Kossel's earlier methods for the isolation and estimation of arginine and has rendered it possible to obtain figures of a hitherto unapproached accuracy for the proportions of arginine yielded by proteins.

Kossel was led to investigate the possibilities of flavianic acid as a reagent for bases by some observations he had made as a young man on the capacity of naphthol yellow-S, the sodium salt of flavianic acid, to stain the nuclei of the sperm cells of fish. The chemical investigation of these sperm cells provided him with a full life-time of valuable work, but as an old man he returned to the study of the staining reaction; the result was a fitting climax to a distinguished career.

IODOGORGOIC ACID (3,5-DIIODOTYROSINE)

$$HO \underset{I}{\overset{I}{\underset{}{\bigcirc}}} CH_2—CH(NH_2)—COOH$$

Iodine has been known, at least since 1848 (264),[13] to be a constituent of certain marine organisms, but its significance in physiology has been appreciated only since the winter of 1895–6 when the reports of two fundamental observations were published. Baumann (251) discovered iodine in the thyroid gland of animals, and Drechsel (253) isolated a new iodine-containing amino acid from the products of alkaline hydrolysis of the horny skeleton of the coral *Gorgonia Cavolinii*. These discoveries led to many investigations in two widely separated fields of biochemistry; recently, however, the isolation of Drechsel's substance from hog thyroids by Harington and Randall (255), and from partially purified thyreoglobulin by Foster (254), has brought these differ-

[13] It was known to Mulder in 1844, since a reference to some investigations of Crookewit on iodine in sponges occurs on p. 329 of Vol. I of Mulder's *Versuch einer allgemeinen physiologischen Chemie*, Braunschweig (1844), translated by H. Kolbe.

ent lines of investigation together and has enormously increased the importance of the curious substance first isolated from a somewhat rare Gorgonian coral.

Drechsel spent a short time in the autumn of 1894[14] at the Marine Zoological Station at Naples and occupied himself with the investigation of a small coral. The axial skeleton appeared to be of a horn-like nature, and he therefore subjected it to hydrolysis with hydrochloric acid, in which it soon dissolved. On continuing the heating, vapors of iodine were evolved from the solution in considerable quantities and in a manner that suggested the gradual decomposition of some unstable iodine-containing substance.

After having satisfied himself of the protein nature of the material by the isolation of lysine, tyrosine, and leucine from the acid hydrolysate, he proceeded to the investigation of the iodine compound. The dry crude protein contained 7.89 per cent of iodine and, since the proportion of iodine was greater than the proportion of ash, the iodine was known to be present in organic combination. No organic iodine compound was known to Drechsel that decomposed in a manner analogous to this substance and, in order to see if the iodine were present as a periodate which was decomposed in the presence of organic matter, he subjected the coral skeleton to hydrolysis with barium hydroxide in the hope that the barium iodate or periodate might be isolated. None could be found. The hydrolysate was therefore freed from barium and treated with silver nitrate. The precipitate which formed was flocculent and partially soluble in dilute nitric acid, and this solution, when boiled a few minutes with a drop or two of strong nitric acid, yielded a copious precipitate of silver

[14] Drechsel's paper (253) appeared in January, 1896, and must, therefore, have been written in 1895. Its first sentence is, "Während der Zeit von Mitte-September bis Mitte October vorigen Jahres hatte ich Gelegenheit, im chemischen Laboratorium der zoologischen Station in Neapel einige Versuche anzustellen." The work was therefore done in 1894. Evidence of the correctness of this is found in Hundeshagen's paper (258) which was published August 15, 1895. In it he mentions a private communication from Drechsel that referred to the isolation from a sea animal of an iodine-containing amino fatty acid, the description of which was shortly to be published.

iodide. The organic iodine compound could therefore be precipitated by silver nitrate and was decomposed by hot nitric acid.

The isolation was effected by precipitation from the alkaline hydrolysate with an excess of silver nitrate; the precipitate was treated with cold nitric acid and filtered from the residue of silver sulfide and iodide and was then neutralized with ammonia. The grey precipitate was decomposed with the minimal necessary amount of hydrochloric acid and the dark solution, on evaporation, yielded an oily residue that slowly deposited crystals. By the careful addition of a little ether a white powdery mass was caused to separate that was filtered off, washed, and recrystallized from hot water. The substance was insoluble in acetic acid but soluble in alkalies and in acids; it formed a crystalline hydrochloride that was hydrolyzed by water; its silver compound was soluble in ammonia and in dilute nitric acid, but was decomposed with the liberation of silver iodide when boiled with excess of nitric acid. Carbon, hydrogen, and iodine determinations were made; these agreed, although very poorly, with the formula $C_4H_8NIO_2$. "Die Formel $C_4H_8NIO_2$ ist die einer Amidojodbuttersäure; bis ihre Constitution sicher erkannt ist, will ich die Substanz einstweilen als Jodgorgosäure bezeichnen."

This was the first organic iodine compound to be isolated from animal material. Drechsel considered it almost certain that the substance was a product of hydrolysis of what he called "ein jodirtes Albuminoid."

There was one important difference between the behavior of the isolated substance and the protein; whereas the latter yielded free iodine on boiling with hydrochloric acid, the crystalline substance was stable. Drechsel pointed out, however, that the instability of the protein might be associated with the presence of oxidizable organic substances during acid hydrolysis of the protein; "vielleicht entsteht ursprünglich aus dem Gorgonin eine Substanz, die der Jodoso oder Jodobenzöesäure V. Meyer's analog zusammengesetzt ist."

Drechsel refers in his paper to a private communication from Hundeshagen in which was described an investigation of the chemical nature of a number of sponges. Hundeshagen had

analyzed many species for iodine and had found several that contained more than 10 per cent of this element.   He had observed the behavior of the sponge skeleton towards acids and alkalies, and had also found that the iodine compound was precipitated by silver nitrate from a neutral solution of the products of alkaline hydrolysis.   He had not, however, been able to isolate it. Hundeshagen drew one very important deduction from his results. This was stated by Drechsel in the following words, "Seine silberhaltigen Lösungen zeigten das beschriebene Verhalten und gaben mit Millon's Reagens eine rothe Färbung; er hält es daher für möglich, dass die jodhaltige organische Verbindung Jodtyrosin ist, und die Substanz der Jodspongien ein jodirtes Spongin."

Hundeshagen published his observations (258) during the long interval that elapsed between Drechsel's investigation in 1894 and the appearance of his paper in 1896.   He mentioned the receipt of a private communication from Drechsel regarding the isolation of iodogorgoic acid.   From the standpoint of date of publication, therefore, Hundeshagen was the first to describe the chemical behavior of the iodine compound derived from the horny skeleton of certain marine animals; Drechsel was the first to isolate it.

It is curious that Drechsel did not follow up Hundeshagen's brilliant suggestion regarding the constitution of the iodine compound.   The analysis of the crystalline preparation was hopelessly inaccurate but it should be remembered that he was a guest in a foreign laboratory and that he accomplished the isolation of cystine from the liver of a dolphin, the isolation of lysine, tyrosine, and leucine, and the discovery of a new amino acid, all in one month.   There was probably little time to check the analysis and, moreover, he had only 0.34 gram of purified substance.

In 1903 Henze (256) took up the study of the protein of *Gorgonia Cavolinii*.   Tyrosine, lysine, and arginine were isolated from this material after acid hydrolysis and its protein nature was placed beyond question.   Henze had difficulty in repeating Drechsel's preparation of iodogorgoic acid but, by a slight modification, finally secured a small specimen.   He pointed out that its formula as a butyric acid derivative was not supported by the analysis of Drechsel; moreover it gave a positive xanthoproteic

test which indicated that it had an aromatic structure; accurate nitrogen and iodine determinations set Drechsel's formula aside at once (nitrogen 3.78 per cent found, 6.11 calculated; iodine 57.3 per cent found, 55.46 calculated). Henze observed that the substance did not give Millon's reaction, but shrewdly noted that *ortho*-substituted tyrosine derivatives likewise fail to give this test.

In 1905 Wheeler, and Jamieson (265) synthesized 3,5-diiodotyrosine from *l*-tyrosine and arrived at the conclusion that their product was identical with those of Drechsel and of Henze. Henze in 1907 repeated their work (257) but could not reconcile the solubility and crystalline form of the synthetic substance with that of iodogorgoic acid derived from coral. It occurred to him, however, that iodogorgoic acid was prepared by alkaline hydrolysis and was optically inactive. He therefore racemized tyrosine, iodated it, and found that the product was identical with iodogorgoic acid in all respects. It is to be noted, however, that the natural substance is almost certainly optically active and derived from *l*-tyrosine; Wheeler and Jamieson's synthetic product, therefore, probably represents the real protein constituent.

Oswald demonstrated in 1909 (261) that the iodine of iodogorgoic acid is frequently extensively removed by enzymatic digestion and in this way resembles the iodine of the globulin of the thyroid gland. "Hiermit ist eine weitere Stütze dafür gewonnen dass das Jod im Schilddrüseneiweiss in ähnlicher Weise gebunden ist, wie im Dijodtyrosin, bezw. an Tyrosin gebunden, wenigstens teilweise, darin vorkommt." Later he succeeded (262) in isolating iodogorgoic acid from an alkaline hydrolysate of artificially iodinated protein thereby demonstrating that a part, at least, of the iodine that can be introduced into proteins attaches itself in the 3,5 position to the tyrosine.

Wheeler and Jamieson (265) had shown that synthetic diiodotyrosine does not give Millon's reaction. Oswald pointed out that this reaction is not given by diiodotyrosine solutions after a considerable part of the iodine has been set free by means of enzymes; he therefore suggested that the effect of the enzyme was to replace the iodine atoms by hydroxyl groups.

Wheeler and Mendel (266) in 1909 isolated iodogorgoic acid from the skeleton of the common sponge and Sugimoto (263), working in Mendel's laboratory, has recently prepared it from the American Gorgonian coral *Plexaura flexuosa*. There is little doubt that this amino acid is widely distributed in marine organisms; the extensive investigations of Hundeshagen (258), of Mendel (259), of Cook (252), and of Mörner (260) have shown that iodine is present in considerable proportions in a great many species. The demonstration that iodogorgoic acid occurs in the protein of mammalian thyroid glands shows that much more importance must now be attached to this amino acid than has formerly been customary.

HISTIDINE

$$CH_2\!-\!CH(NH_2)\!-\!COOH$$

$$\begin{array}{c} C\!-\!NH \\ \| \qquad\quad \diagdown CH \\ C\!-\!-\!N \diagup \end{array}$$

In 1874 Miescher (274) observed that a large proportion of the dry weight of the sperm of the Rhine salmon consisted of a "nuclein" that was combined, in the sperm, "in einer unlöslichen, salzartigen Verbindungen mit einer organischen Base, dem Protamin." This basic substance could be secured from an acid extract of the material by precipitation with chloroplatinic acid or by mercuric nitrate. The new base contained a high proportion of nitrogen, the ratio of nitrogen to carbon atoms being approximately 5:9. Miescher was chiefly interested in the physiological aspects of the problem and he therefore invited Piccard to continue the investigation. Piccard (276) found that Miescher's original preparation of the chloroplatinate probably contained guanine and hypoxanthine; he also suggested improvements in the method to obtain protamine.

No further investigation of this substance was made until 1894, when Kossel observed that protamine yielded a precipitate with solutions of soluble proteins and that these precipitates had a number of properties in common with the histones. Kossel (272)

followed up this observation and, in the search for a more convenient source of protamine than the Rhine salmon, he investigated the sperm of the sturgeon.   An analogous basic substance was found in this material; the meaning of Miescher's term protamine was therefore extended to cover the class of substances and the specific designations sturine and salmine were applied to the two individuals then known.

The characteristic property of these substances was that, after hydrolysis with strong acid, a large part of the products of hydrolysis could be precipitated by phosphotungstic acid.   Protamines therefore yield a large proportion of strong organic bases.   Kossel subjected sturine to hydrolysis with sulfuric acid, removed the reagent quantitatively with barium hydroxide, and added mercuric chloride to the strongly alkaline solution.   The precipitate "enthält eine bisher unbekannte Base, für welche ich die Namen Histidin vorschlage" ($\iota\sigma\tau\acute{o}s$, tissue).   The chloride of the new base, on analysis, gave results that agreed either with $C_{12}H_{20}N_6O_4 \cdot 2HCl \cdot 2H_2O$ or with $C_6H_9N_8O_2 \cdot HCl \cdot H_2O$.   The determination of the molecular weight gave a figure intermediate between the requirements of these formulas.   The free base crystallized readily from mixtures of water and alcohol, or even from water; its aqueous solution was alkaline.   Measurements of the crystals of the chloride were carried out by Bauer and were communicated to Kossel in October 1895.

The results of this work were reported in the proceedings of the Prussian Academy of Science on April 9, 1896.   On May 11, 1896 the editors of the Zeitschrift für physiologische Chemie[15] received a paper from S. G. Hedin (268) in which the isolation of a new base from protein hydrolysates was described.   Hedin, in the course of his work on arginine, had accumulated a considerable amount of the amorphous precipitate which formed when silver nitrate was added to a solution of the free bases derived from proteins.   This precipitate was decomposed by hydrogen sulfide and a solution was secured that yielded a non-crystallizing sirup when concentrated.   It contained, as an impurity, a substance that

[15] Baumann and Kossel.   Baumann died November 5, 1896.

liberated hydrogen sulfide when heated with alkali. The lead-blackening sulfur compound could be removed by precipitation with lead acetate and ammonia, and the filtrate, after removal of lead, was treated with silver nitrate. This solution was, of course, acid with acetic acid derived from the lead acetate and no precipitate separated. Careful addition of ammonia, however, brought down a voluminous amorphous precipitate which was soluble in excess of this reagent.

It is to be noted that Hedin employed at the very first the method which has since been shown to be by far the best for the isolation of histidine. The silver precipitate was decomposed by the minimal amount of hydrochloric acid, silver chloride was removed and, when the solution was decolorized and evaporated, a crystalline chloride separated which gave an analysis in excellent agreement with the formula $C_6H_9N_3O_2 \cdot HCl \cdot H_2O$. The free base was secured by the use of silver sulfate and was crystallized from aqueous alcohol in plates and needles that dissolved in water to form a very faintly alkaline solution. The results of an analysis agreed with the formula $C_6H_9N_3O_2$ and a determination of the molecular weight by the freezing point method gave 155.4 (theory, 155). The material was obviously purer than that of Kossel, who reported that the aqueous solution of the new base was strongly alkaline. This discovery was a logical outgrowth of Hedin's previous work on arginine and it was the merest chance that Kossel's brilliant discovery should have preceded his by only a few weeks. Hedin pointed out that the new base corresponded very closely in composition with a preparation secured by Siegfried (279) when he first encountered the amorphous silver precipitate. Siegfried had prepared a chloride from this material that gave figures, on analysis, corresponding to $C_{11}H_{20}N_6O_6 \cdot 2HCl$. If this formula were written $C_{12}H_{22}N_6O_6 \cdot 2HCl$, as it might have been without doing too much violence to the analytical results, and then halved to $C_6H_{11}N_3O_3 \cdot HCl$, a formula is obtained that differs very little from the correct one suggested by Hedin.

The new base was obviously closely related to the base histidine described by Kossel and, in a paragraph at the end of his paper, Hedin referred to the close similarity of the measurements of the

crystal angles of the chlorides of the two preparations. There were small differences, however, in the ultimate composition and in the behavior, which prevented complete identification of the two.

The simultaneous discovery of histidine in two widely separated laboratories in the course of work that originated from two totally different lines of research is of great interest. Hedin hit upon a method of isolation.better than that of Kossel; his preparations were purer and his analytical work was superior. Moreover, the investigation was the logical development of observations on the behavior of protein hydrolysates with silver nitrate made originally by his fellow-worker Siegfried five years before and repeatedly encountered in his own work. Kossel on the other hand was investigating a wholly new field. This was his first paper on the subject with which his name is now most closely linked. That he should immediately apply severe hydrolysis for the decomposition of the new protamine was genius; his use of mercuric chloride was, however, largely conventional and, under the circumstances, he was extremely fortunate to secure material as pure as he did. Mercuric chloride is far from being a selective reagent in alkaline solutions, but the products of hydrolysis of a protamine contain so few amino acids in addition to arginine that the precipitate could have contained little except histidine. The final identification of the bases prepared by Kossel and by Hedin was made by Kossel and Kutscher (273).

Kossel and Hedin deserve equal credit as the independent discoverers of histidine; each so regarded himself. Kossel assumed the right to name his substance in 1896. Hedin (269) in 1898, in referring to the three bases derived from proteins wrote, "Da die ebene genannten Basen von Drechsel (Lysin) und von mir (Arginin und Histidin) unter den Spaltungsprodukten aller . . . . Proteinstoffe gefunden waren . . . ." etc. Perhaps the fairest assignment of credit is to regard Kossel as the discoverer of histidine in protamines and Hedin as the discoverer of histidine in proteins.

The wide distribution of histidine became apparent from the work of Kossel and his students and from that of Schulze (278).

Its constitution was not ascertained for some years. It was soon found that it was a diacid base and that it contained an asymmetric carbon atom. In 1903 Herzog (270) observed that histidine responds to the biuret test, that it contains no methyl or oxymethyl group, that it withstands oxidation in acid solution, although in alkaline solution it yields hydrocyanic acid, ammonia, and carbon dioxide, and that it can not be brominated. At about the same time Fränkel (267) showed that it contains a carboxyl and an amino group and gives Weidel's pyrimidine reaction. He suggested a constitution based on the pyrimidine ring but, since his formulation contained no asymmetric carbon atom, it was not accepted. Pauly in 1904 took up the problem at Kossel's request (275). He verified the presence of a carboxyl group by preparing a methyl ester. He showed that two equivalents of naphthalenesulfonyl chloride could be introduced, consequently histidine contained one secondary amine group in addition to the primary amine group; the third nitrogen atom must therefore be tertiary. The stability of histidine towards oxidation, and the presence of two hydrogen atoms replaceable by silver, suggested that a ring structure was present. Of the possible structures the imidazole ring seemed by far the most probable. Imidazole derivatives react with diazobenzenesulfonic acid to form highly colored products. Pauly found that histidine behaved in this way, and histidine, which had previously been written $(NH_2) \cdot C_5H_6N_2 \cdot COOH$ was therefore formulated,

$$
\begin{array}{l}
CH\text{---}N \\
\quad\quad\quad\quad\diagdown \\
\| \quad\quad\quad\quad\quad CH \\
\quad\quad\quad\quad\diagup \\
C\text{---}NH \\
| \\
CH_2 \\
| \\
CHNH_2COOH
\end{array}
$$

The formula contained an asymmetric carbon atom and, in fact, accounted for every known reaction of histidine. Knoop and Windaus (271) shortly afterwards confirmed this view by the demonstration that histidine, after treatment with nitrous acid to remove the α-amino group, could be reduced to β-imidazol-

propionic acid identical with the synthetic product.   This conversion showed that Pauly was essentially correct, the only point remaining undetermined being the position of the primary amino group.   This was finally proved to be an α-amino group by Pyman in 1911 (277) who synthesized racemic histidine from diaminoacetone.   The imidazole ring was formed by condensation with potassium thiocyanate followed by oxidation with nitric acid.

$$
\begin{array}{ccc}
\mathrm{CH_2-NH_2} & \mathrm{CH-NH} & \mathrm{CH-NH} \\
| & \| \quad \diagdown \mathrm{C-SH} & \| \quad \diagdown \mathrm{CH} \\
\mathrm{CO} \longrightarrow & \mathrm{C-\!-\!-N} \diagup & \mathrm{C-\!-\!-N} \diagup \longrightarrow \\
\mathrm{CH_2-NH_2} & \mathrm{CH_2-NH_2} & \mathrm{CH_2OH}
\end{array}
$$

The hydroxymethylglyoxaline was converted to the chloride with phosphorus pentachloride and this intermediate served for the synthesis of histidine by the sodium malonic ester method. Resolution of the racemic product was effected by means of d-tartaric acid.

<div align="center">

PROLINE

$$
\begin{array}{cc}
\mathrm{CH_2-\!\!-\!\!-CH_2} & \\
| \qquad\quad | & \\
\mathrm{CH_2} \quad \mathrm{CH-COOH} & \\
\diagdown \qquad \diagup & \\
\mathrm{NH} &
\end{array}
$$

</div>

Proline is the second amino acid to be obtained synthetically before its presence as a product of the hydrolysis of the protein molecule was recognized.   It was first prepared in 1900 by Willstätter (292), who was interested in the position of the carboxyl group in hygric acid.   This substance is obtained by oxidizing (289) hygrine and cuscohygrine, alkaloids found by Liebermann (287) in Peruvian cusco leaves.   They are closely related to cocaine and tropa-cocaine.   The question arose as to whether the carboxyl group in hygric acid (N-methylpyrrolidine-α-carboxylic acid) was in the α or the β position.   From the fact that carbon dioxide was easily split off by dry distillation, Liebermann and Cybulski (288) were inclined to favor the view that it was in

the α position.   The position of the carboxyl group would determine whether hygrine and cuscohygrine were related to the tropane group of alkaloids which are α_1 and α_2 substitution products of N-methylpyrrolidine.

Willstätter condensed sodium malonic ester with trimethylenebromide and obtained bromopropylmalonic ester.   By treating this product with bromine in the cold it was converted into α,δ-dibromopropylmalonic ester.   This compound was then treated with ammoniacal methyl alcohol and was converted into an amide which, when saponified with barium hydroxide, yielded α-pyrrolidinecarboxylic acid.   By treating dibromopropylmalonic ester with methylamine and subsequently saponifying, Willstätter obtained a small yield of N-methylpyrrolidinecarboxylic acid which proved to be identical with Liebermann's hygric acid.   Thus, in an unexpected way, a protein amino acid was discovered.

A year later Fischer (280) published a synthesis of proline from phthalimide propylmalonic ester.   This compound was treated with bromine and converted into phthalimide propylbromomalonic ester.   By treating the latter substance with ammonia, Fischer had hoped to prepare a derivative of α,δ-diaminovaleric acid.   Instead there was obtained a mixture of phthalimide and other products.   On heating with hydrochloric acid at 100° he obtained α-pyrrolidinecarboxylic acid.   Although this work appeared a year after Willstätter's publication, it had been begun without the knowledge of the latter's results.   Fischer states, "Ich bemerke übrigens dass meine Versuche längst begonnen waren, bevor die Arbeit des Herrn Willstätter zu meiner Kenntniss kam."

More recently methods of synthesizing proline have been described by Sörensen and Andersen (291) and by Fischer and Zemplén (286).

Proline was first isolated from a protein by Fischer in 1901 (281).   He hydrolyzed casein with hydrochloric acid, esterified the amino acids and distilled the esters.   The fraction boiling at 65–80° was saponified and subjected to fractional crystallization.   The mother liquor, resulting from one of these fractions, was

boiled with copper oxide and yielded the copper salt of racemic proline together with small amounts of other amino acids. This product was decomposed with hydrogen sulfide, extracted with alcohol, again converted into the copper salt and subsequently freed from copper. Analysis of the final product gave results which corresponded to pyrrolidinecarboxylic acid. Racemic proline copper crystallizes well from water but l-proline copper remains in the mother liquor and yields an amorphous mass when this is evaporated. Fischer devised the artifice of racemizing the free acid, prepared from this material, by treatment with barium hydroxide at 140–145°. The racemic copper salt could then be readily crystallized. Fischer prepared crystalline proline from alcohol and ether mixtures or from water with the aid of a little pyridine. His early preparations melted at 203–206° uncorrected; later, however, the pure acid of melting point 220° was obtained.

The most important problem in the early stage of the investigation was to obtain a demonstration that proline was a primary product of protein hydrolysis. There was a possibility that it might be formed by ring closure from some other amino acid. Fischer first attempted to get proline from arginine by subjecting the basic amino acid to the procedure used in preparing proline. Ornithine was likewise investigated without result. Together with Levene (281) he investigated a tryptic digest of casein and secured a small quantity of proline. In this case, however, the amino acid had been exposed to the action of acid during esterification and there was a possibility that this may have given rise to ring closure although Fischer regarded it as improbable. In 1902 Fischer reported (282) the isolation of proline from casein that had been hydrolyzed by alkali.

The final proof that proline is a primary product of protein hydrolysis was secured by Fischer and London (284), who isolated nearly as much proline from an enzymatic digest of gliadin as had been obtained by acid hydrolysis.

Proline is closely related to glutamic acid. If the latter acid is boiled with water ring closure occurs, and an equilibrium mixture of pyrrolidonecarboxylic acid and glutamic acid results. When this mixture is heated with acid the ring opens and glu-

tamic acid is again formed. The possibility that proline can be formed in nature by the reduction of the keto group in pyrrolidonecarboxylic acid is of great importance. Experimentally, however, this reduction is very difficult. It was accomplished by Fischer and Boehner in 1911 (283) although with small yields. A further extensive investigation of this subject was carried out in 1926 by McCay and Schmidt (290) with negative results.

Fischer (285) first suggested the use of the word proline to designate α-pyrrolidinecarboxylic acid in connection with his work on the synthesis of prolylalanine. An excerpt from his paper states his reason for the use of the term. "Für die Benennung derartiger Kombination ist das Wort α-Pyrrolidincarbonsäure zu lang. Wir halten es deshalb zweckmässig, das abgekürzte Wort 'Prolin', dessen Ableitung aus Pyrrolidin leicht verständlich ist, vorzuschlagen."

TRYPTOPHANE

The story of tryptophane, during the early years of protein chemistry, is the story of a color reaction. The literature contains innumerable references to more or less intense color reactions produced by the action of a wide variety of reagents on proteins or on their decomposition products. One of the earliest of these was described by Tiedemann and Gmelin (315)[16] in their

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[16] The first edition of this extraordinary book appeared in 1826. It was an essay presented in 1825 to the French Academy in competition for a prize; the subject assigned by the Academy was "quel sont les phénomènes qui se succèdent dans les organes digestifs durant l'acte de la digestion. . . . . Les expériences devront être suivies dans les quatre classes d'animaux vertébrès." None of the essays was regarded by the Academy as worthy of a prize, but two were given honorable mention and an award of 1500 francs "à titre d'encouragement." The two essays selected were those submitted respectively by Leuret and Lassaigne and by Tiedemann and Gmelin. Tiedemann and Gmelin wrote to the Academy that their work "ne l'ayant pas trouvé digne du prix, nous ne pouvons accepter, ni la mention honorable, ni la recompense de 1500 francs. . . . . Nous ne tarderons pas, à soumettre nôtre travail au jugement impartial du monde savant."

elaborate investigation of the digestive processes of vertebrate animals. Pancreatic juice secured from a dog was evaporated to dryness and the residue was extracted with alcohol. The extract was in turn evaporated and the residue was dissolved in water and filtered. "Wenig wässriges Chlor färbte die Flüssigkeit lebhaft rosenroth, und schlug nach 12 Stunden zarte violette Flocken nieder, wobei sich die Flüssigkeit fast gänzlich entfärbte. Eine grössere Menge von Chlor zerstörte augenblicklich, ohne alle Trübung, die rothe Farbe, welche durch eine geringere Menge bewirkt war." A similar color test was obtained on the fluid found in the small intestine of a calf, of a hen, of a frog, and of a trout. In connection with the observation on the calf they wrote, "Ob diese Materie mit derjenigen einerlei ist, welche bei der Destillation des Inhaltes von verschiedenen Magen und andern Theilen des Darmkanals der Wiederkauer bei der Destillation übergieng, und beim Abdampfen derselben mit Salzsäure sich durch Röthung der Flüssigkeit zu erkennen gab, ist nicht ausgemacht, doch unwahrscheinlich."

Claude Bernard (295, p. 403–409) showed that a mascerate of the pancreas does not give the color reaction with chlorine water until putrefaction sets in. After putrefaction has been allowed to go on for some time the color reaction can no longer be obtained. Mascerates of liver, spleen, and certain other glands gave the reaction under similar circumstances. "Il semblerait que cette matière rouge existe dans les organes qui agissent chimiquement dans la vie de nutrition et non dans les appareils de la vie de relation . . . . . Enfin nous ajouterons que le tissu pancréatique, après avoir été bouilli, perd la propriété de donner une infusion susceptible de rougir par le chlore." Bernard made no attempt to account for the chemistry of this reaction but drew the following deduction (p. 406): "Nous dirons seulement que cette matière, qui a la propriété de se colorer, semble appartenir aux substances protéiques analogues à la caséine"; and again when referring to experiments on pure pancreatic juice (p. 433), "Cette matière colorante rouge, qui est la même qui se forme aussi dans le tissu pancréatique, se produit dans le suc pancréatique par suite de la décomposition de la matière organique coagulable contenue dans ce liquide."

Stadelmann (314), in quoting Bernard's paper in 1890, stated that Bernard had observed that the color reaction was given by a tryptic digest of casein. This statement appears, however, to be an error, although it has been widely quoted. Bernard's words are as quoted above; he may have tried the experiment but does not specifically say so. Curiously enough Bernard was not able to obtain the color reaction with either bromine or iodine (p. 434). Kühne, however, introduced bromine water as a reagent for the test (306), and it has now entirely replaced chlorine water for this purpose. Bernard was correct, however, with respect to iodine.

Kühne showed that indole is not produced during properly conducted tryptic digestions of protein; it is formed, however, if putrefaction occurs and this observation was confirmed by Nencki (309). The association of indole with the tryptophane reaction was due to the observation of Bernard that, after extensive putrefaction of pancreas, a substance is present that gives a violet color reaction with nitric acid; this substance he believed to be the same as that responsible for the reaction with chlorine water. Kühne identified the former substance as indole, and thus indole and tryptophane very early became associated with each other.

Neumeister (312) in 1890 found that "Das fragliche Chromogen lässt sich bei allen Processen, welche den tiefen Zerfall der Eiweisskörper herbeiführen, nachweisen und kann daher wohl als 'Tryptophan'[17] bezeichnet werden. Man beobachtet sein Auftreten ausser bei der Pankreasverdauung auch bei andauernder Fäulniss, beim Erhitzen der Eiweisskörper mit Barytlauge und beim Kochen derselben mit 5% Schwefelsäure." He found that the substance was stable in boiling water. Neumeister also made the observation, "Auch das Tryptophan selbst wird vom Amylalkohol aufgenommen und lässt sich hierdurch seinen Lösungen entziehen. Da Leucin und Tyrosin in Amylalkohol unlöslich sind, könnte das Tryptophan vielleicht auf diese Weise isoliert werden." In view of Dakin's introduction in 1918 of the use of butyl alcohol for the convenient preparation of tryptophane, this statement is extraordinarily interesting.

[17] The name is derived from θρύπτομαι, to be broken, and φαίνω, to bring to light.

A number of workers became interested in the tryptophane reaction about 1890 and the large tryptophane literature of this period indicates the importance that was attached to it. Stadelmann as well as Neumeister suggested a name for the color-producing substance (314): "Ich werde von der ursprünglichen Substanz, die also bei der tryptischen Verdauung z.B. direct entsteht, als dem Proteïnchromogen und von der Brom- resp. chlor-Verbindung die eben einen violetten rothen Körper darstellt als dem Proteinochrom sprechen." Gamgee (302) objected to this name because it implied that no other color-producing substance was present in the protein; the word was too inclusive in its implications. Neumeister's convenient and non-committal name was preferred by many and the investigations became centered around the constitution of the colored product of the action of halogens. Nencki (311) prepared the colored substance and observed the formation of indole and skatole from it after fusion with alkali. Beitler (294) attempted to isolate both colored substance and precursor but failed to obtain pure material. Kurajeff (307), however, obtained products that suggested the presence of an indole derivative that contained two nitrogen atoms. In Hopkins' opinion this worker had isolated a product which may have been a monobromo derivative of tryptophane.

Another striking color reaction of proteins was observed in 1874 by Adamkiewicz (293) when glacial acetic acid was mixed with a solution of albumin and the mixture was treated carefully with concentrated sulfuric acid. This reaction was studied by Hopkins and Cole in 1901 (303) who showed that the formation of the color was due to the presence of glyoxylic acid in the acetic acid employed. With this color test and the well-known tryptophane reaction as guides, they then took up the problem of isolating the chromogenic substance in protein digests (304).

Hopkins and Cole noticed that the glyoxylic acid reaction was given by an acid hydrolysate of proteins long after the biuret reaction had disappeared; it was evident that the chromogenic substance survived hydrolysis and it was therefore, probably, a relatively simple substance. They further observed that the

reaction was exceptionally intense when applied to the products of a tryptic digest that gave no biuret reaction. Enzymatic digestion, consequently, seemed a more suitable method for hydrolysis of the protein. Casein was therefore treated with an active preparation of pancreatin until the tryptophane reaction with bromine water attained maximal intensity.

Beitler had shown that none of the more usual reagents could be successfully used for the precipitation of the chromogenic substances from protein digests. Hopkins and Cole found, however, that mercuric sulfate, a reagent that had previously been used but little for chemical separations, had a special selective action on the substance responsible for the tryptophane reaction, when added to a strongly acidified solution. "From the final products of proteid hydrolysis it throws down, in appreciable quantity, only cystine and the substance which forms the subject of this paper." The separation of cystine from the other product was easy, as it could be precipitated first by the addition of a small amount of reagent. The chromogenic substance could then be isolated by a second mercuric sulfate precipitation.

The final procedure adopted by Hopkins was relatively simple. The clear tryptic digest was acidified with 5 per cent by volume of sulfuric acid and the mercuric sulfate reagent was added in an amount of roughly 1 gram of mercuric sulfate per gram of protein digested. The yellow precipitate was removed, after from 12 to 24 hours, and washed free from tyrosine with 5 per cent sulfuric acid; it was then decomposed with hydrogen sulfide and the solution was again acidified by the addition of 5 per cent of sulfuric acid. The next step was the removal of cystine; mercuric sulfate was carefully added until a small permanent precipitate was produced. The solution was allowed to stand for half an hour and was then filtered; the precipitate so secured contained all but traces of the cystine. Excess of reagent was then added to the filtrate and the precipitate was removed after a few hours and decomposed with hydrogen sulfide. The sulfuric acid contained in this solution, derived from the decomposition of the mercuric sulfate compound, was exactly removed with baryta and the fil-

trate was mixed with alcohol.   It is at this point that difficulty was encountered.   It was found that the evaporation of the solution must be carried out with continued additions of alcohol as, otherwise, the product undergoes extensive decomposition. The alcohol concentration at the end should be over 60 per cent. After proper evaporation and cooling the solution deposited a magma of crystals that were filtered off and recrystallized from 75 per cent alcohol.   ·

The new substance gave the tryptophane and glyoxylic acid reactions with great intensity.   It responded to the pyrrole pine-splinter reaction and, when heated, gave off vapors of indole and skatole.   The analysis agreed closely with the formula $C_{11}H_{12}N_2O_2$. The colored product of the action of bromine on the substance gave an absorption spectrum identical with that observed in the case of the tryptophane reaction.   Hopkins and Cole therefore considered that it was desirable "that the new compound, which is the mother substance of the most characteristic coloured product, should continue to receive Neumeister's designation of tryptophane."

Nencki (308, 309, 310), Kühne (306), and also Salkowski (313) had found indole, skatole, skatolecarboxylic and skatoleacetic acid among the products of the putrefaction of proteins.   Nencki in 1889 arrived at the conclusion that the mother substance of these substances might be skatoleaminoacetic acid.

Skatoleaminoacetic acid

The new substance of Hopkins and Cole likewise yielded these four products and they therefore regarded their new substance as a derivative of skatole.   Ellinger (301, 296), however, found that tryptophane could behave as the precursor of indole in the intestine and the accuracy of Nencki's formula was therefore

called in doubt.   Ellinger synthesized indole-3-acetic acid (297) and found that it was

$$\text{I—C—CH}_2\text{—COOH}$$

Indole-3-acetic acid

identical with the substance isolated by Salkowski.   Tryptophane must therefore be an indole rather than a skatole derivative and must have one of the constitutions,

(I) $\text{I—CH}_2\text{—CH(NH}_2\text{)COOH}$

(II) $\text{I—CH(NH}_2\text{)CH}_2\text{—COOH}$.

(III) $\text{I—CH(COOH)CH}_2\text{—NH}_2$

(IV) $\text{I—C(NH}_2\text{)(CH}_3\text{)—COOH}$

Formula III was at first regarded as most probable but the synthesis of indole-3-propionic acid (298) and the demonstration that the product was identical with Nencki's skatoleacetic acid showed that tryptophane must possess either formula I or II. The first was more probable, owing to the analogy with other amino acids.   Hopkins and Cole (305) had obtained a substance C_9H_7NO, by the oxidation of tryptophane with ferric chloride, which Ellinger (299) showed to be β-indolealdehyde.   Using this compound as a starting point Ellinger and Flamand in 1907 (300) synthesized tryptophane by the hippuric acid condensation method and showed that its constitution is represented by formula I.

OXYPROLINE

$$\text{HO—CH——CH}_2$$
$$\text{CH}_2 \quad \text{CH—COOH}$$
$$\text{NH}$$

Thirty-seven years after the discovery of serine in silk gelatin by Cramer (316), Fischer and Skita (318) published a paper in

which they showed that serine was a constituent of fibroin.   In referring to this discovery Fischer and Skita (318) wrote, "Wir glauben diese Beobachtung besonders hervorheben zu dürfen, da das Serin, welches zurzeit noch die einzige natürliche Oxy-aminosäure der aliphatischen Reihe ist, bisher nur im Seidenleim aufgefunden wurde, und wir machen von neuem darauf aufmerksam, dass diese Oxyaminosäuren eine bis jetzt gar nicht gewürdigte grosse .Bedeutung für das Studium der Proteïne haben."   Four months later, Fischer (317) published a paper in which he described the isolation of oxyproline from gelatine. He stated, "Ich bin ferner überzeugt, dass kohlenstoffreichere Oxyaminosäuren noch in grösserer Zahl unter den Spaltungsproducten der Eiweisskörper vorhanden sind, denn es ist mir gelungen, eine derselben, von der Formel $C_5H_9O_3N$ aus dem Leim zu isoliren."

Gelatin was hydrolyzed and the esters were prepared and liberated from their acid salts in the usual manner.   After extraction with ether there remained a residue which contained inorganic salts, and the basic amino acids, together with residues of the monoamino acids.   The salts were eliminated by extracting the residue with acidified alcohol, which dissolved the amino acids, and these were again subjected to the esterification process. After a second extraction with acid alcohol the hydrochloric acid was removed with silver and the basic amino acids were precipitated with phosphotungstic acid.   The excess of the reagent was removed with barium hydroxide in the usual manner and, after concentrating *in vacuo*, crystals of a new amino acid were obtained.   By heating with hydriodic acid and phosphorus, the new substance was converted into proline.   Although he had no absolute proof, Fischer surmised that the new amino acid was oxyproline.   "Jedenfalls kann man aus dem Resultat des Versuches den recht wahrscheinlichen Schluss ziehen, dass die neue Aminosäure eine Oxypyrrolidine-α-carbonsäure ist.   Leider liegt bisher keine Beobachtung vor welche ein Urteil über die Stellung des Hydroxyls gestattete."

The synthesis of oxypyrrolidinecarboxylic acid was first carried out by Leuchs (320) who, following the precedent of Fischer with

respect to proline, named the compound oxyproline. Epichlorhydrin and sodium malonic ester were condensed to α-chloro-β-oxypropylmalonic ester. By splitting off alcohol, this compound was converted into the lactone. The lactone was then converted into α-bromo-δ-chloro-γ-valerolactone by bromination. On treating with ammonia, r-oxyproline was formed; when treated with hydriodic acid, this was reduced to proline.

Leuchs and Felser (322) and Leuchs and Bormann (321) have attempted to place the position of the hydroxy group in the pyrrole ring. On heating natural oxyproline with barium hydroxide at 200°, it was only partially racemized, indicating that in oxyproline there are two asymmetric carbon atoms present. They believed that the oxyproline occurring in proteins is either γ- or β-oxyproline but their experiments do not differentiate as to which of these two possibilities is correct.

Hammarsten (319) has synthesized γ-oxyproline but furnished no comparison between his preparation and natural oxyproline. His preparation melted at the same temperature as that of Leuchs.

The difficulty of deciding the exact constitution of oxyproline arises from the presence of the second asymmetric carbon atom in the ring. This atom is apparently not racemized by ordinary treatment and, consequently, the decision will have to await the preparation of the oxyprolines derived from the four optically active isomeric intermediates. In view of the fact that at least eight optically active isomeric oxyprolines are possible it is perhaps not surprising that the synthesis has not yet been accomplished.

<div align="center">ISOLEUCINE</div>

$$\begin{array}{c} CH_3 \\ {}\diagdown \\ {}CH-CH(NH_2)-COOH \\ {}\diagup \\ C_2H_5 \end{array}$$

Emil Fischer in 1901, when investigating the separation of the individual amino acids of the leucine fraction secured by the ester distillation method (327), found that successive crops of crystals of different specific rotations were obtained. The least soluble

fraction had a lower, but the second and other fractions all had a higher rotation than that described by Schulze for l-leucine.   He wrote, "solange dieser Wert nicht als zu niedrig erkannt ist, muss man annehmen, dass in der Fraction D und höchst wahrscheinlich auch in B und C eine gleich zusammengesetzte, aber stärker drehende Aminosäure enthalten ist."   Attempts to separate this substance from leucine failed.

The explanation of this behavior came from a brilliant investigation of the nitrogenous substances in beet-sugar molasses carried out by Felix Ehrlich in 1903 (324).   This material was customarily evaporated to a thick sirup, previous to its distillation for the recovery of trimethylamine and ammonia, and of the potassium salts.   The sirup, on standing, deposited a crystalline mass, more than half of which frequently consisted of organic substances.   Ehrlich filtered this material and extracted the residue with hot alcohol.   The extract, when evaporated, deposited a material that showed the properties of a mixture of amino acids. He observed that the product was soluble in alcohol that contained a little ammonia; by the use of this reagent he succeeded in obtaining a fairly pure crude leucine in a yield of 1 to 2 grams per kilogram of sirup.   This, when recrystallized yielded the gleaming plates typical of pure leucine and gave results, on analysis, that agreed with the formula of leucine.   Its properties, however, were somewhat different from those described by Schulze for pure l-leucine.   It was slightly less soluble in water, its specific rotation in 20 per cent hydrochloric acid was somewhat higher and the melting point was lower.   The most striking difference lay in the solubility of the copper salt.   Pure l-leucine copper is very insoluble in water or in alcohol, although when mixed with valine the mixed copper salts are much more soluble.   Ehrlich's material, however, gave two copper salts, one insoluble and the other much more soluble in water.   Furthermore the benzene-sulfonyl chloride derivatives of the leucines obtained from the two copper salts differed in melting point.   "Die weitere Untersuch-ung zeigte nun, dass die . . . . . Substanz trotz ihrer grossen Aehnlichkeit mit dem l-Leucin nicht einheitlich war, sondern aus einem Gemisch von r-Leucin, l-Leucin und einem Isomeren des

Leucins, dem *d*-Isoleucin, bestand." This explanation was obtained by investigating the alcoholic mother liquors from which the preparation had been recrystallized. These yielded a preparation with the composition of leucine that had a specific rotation of $[\alpha]_D^2 = +28.1°$ (whereas *l*-leucine has $[\alpha]_D^{20} = +17.3°$) in 22 per cent hydrochloric acid and which was weakly dextrorotatory in water (*l*-leucine is levorotatory); it was soluble in only 30 parts of water at 19°. This material was obviously entirely different from Schulze's *l*-leucine. Similar preparations were secured from the more soluble fractions of the copper salt. Although no homogeneous product had been obtained it was evident that the material in hand was a mixture of *l*-leucine with a dextrorotatory substance of the same composition and therefore probably an isomer.

At this point Ehrlich made the brilliant observation that the copper salt of the isomeric leucine was soluble in methyl alcohol and could be partially extracted from the mixed copper salts with this solvent. He was therefore able to secure relatively pure specimens. Only a part of the isoleucine present could be secured by a single extraction; it was then necessary to decompose the insoluble copper salt, reconvert it to the copper salt and extract again, and further repetition of these operations was frequently necessary. The new substance, when crystallized from water and alcohol, usually separated in leaves indistinguishable from ordinary leucine. By slow evaporation of a solution that was only slightly supersaturated it was obtained in tablets and prisms. The specific rotation in water was +9.74° and in 20 per cent hydrochloric acid was +36.8°; the copper salt crystallized in leaves differing in appearance from *l*-leucine and, unlike this, was soluble in methyl and benzyl alcohol. The benzoyl and other similar derivatives differed in melting point from the compounds of *l*-leucine described by Fischer.

In order to settle the question of the origin of this substance, Ehrlich next prepared crude leucine from a pancreatic digest of fibrin. This material likewise gave a copper salt, part of which was soluble in methyl alcohol, and from this fraction he prepared isoleucine that corresponded in properties with the preparation

from beet molasses. It was therefore clear that isoleucine was a product of protein decomposition and not an artifact produced during the industrial processes through which beet-sugar molasses had passed. He also secured specimens from egg albumin, wheat gluten and beef muscle. These observations cleared up most of the discrepancies that had been observed in the behavior of leucine from proteins. Ehrlich later showed that the original isoleucine preparation obtained from fibrin contained traces of valine and pointed out the great difficulty of separating isoleucine from this substance. The success of the early preparations from beet molasses was due to the absence of valine from this material.

Ehrlich continued the investigation of isoleucine and in 1907 (325) obtained evidence of its constitution. Isoleucine and leucine crystallize together in mixed crystals and it is impossible to separate the two substances by any process of crystallization; furthermore the two amino acids are almost always found together in nature. Ehrlich pointed out that there was one other pair of well-known substances, isoamyl alcohol and d-amyl alcohol that likewise always occurred together and that all of their derivatives formed similar series of mixed crystals. "In der Tat liess sich nun zeigen, dass die so auffallend übereinstimmenden Eigenschaften dieser beiden Verbindungspaare keine zufälligen sind, sondern dass zwischen den beiden Leucinen und Amylalkoholen sehr nahe chemische Beziehungen bestehen und eine weitgehende Analogie, die besonders für die Frage der Entstehung des Fuselöls bedeutungsvoll geworden ist."

It had long been known that when isoleucine was heated to 200° isoamylamine of the constitution

$$(CH_3)_2=CH-CH_2-CH_2-NH_2$$

was produced. Isoleucine, when treated the same way, gave an optically active amylamine that was identical with the d-amylamine

$$\begin{matrix} CH_3 \\ \diagdown \\ \diagup \\ C_2H_5 \end{matrix} CH-CH_2-NH_2$$

synthesized by Marckwald (328), except that the optical activity was slightly lower. This indicated that *d*-isoleucine must be one of the four possible optical isomers of the constitution

$$CH_3 \diagdown CH-CH(NH_2)-COOH$$
$$C_2H_5 \diagup$$

This view was supported by the result of fermenting isoleucine with yeast in the presence of sugar since a *d*-amyl alcohol was obtained. This was proved by oxidation of the alcohol to the dextrorotatory valeric acid identical with methylethylacetic acid. A synthesis was therefore attempted starting with *d*-amyl alcohol. This was oxidized to *d*-valeraldehyde and converted to the amino acid by Strecker's method. The product yielded a copper salt soluble in methyl alcohol, but the free acid was different in some respects from *d*-isoleucine. Isoleucine contains two optically active carbon atoms and, since the synthesis started with *d*-amyl alcohol, the product should be a mixture of the two isomers produced by the introduction of the second asymmetric carbon atom. One of these should be *d*-isoleucine, the other was named allo-isoleucine. A similar mixture of isomers was produced by heating *d*-isoleucine with barium hydroxide under pressure. No means of separating these substances was found, but pure allo-isoleucine was secured by fermentation of the mixture for this substance was not utilized by the yeast. The preparations of allo-isoleucine secured in this way from synthetic material and from isoleucine were identical.

A complete synthesis of isoleucine was carried out by Bouveault and Locquin in 1906 (323) starting with *secondary*-butyl aceto-acetic ester and still another by Ehrlich (326) who started with *secondary*-butyl iodide and used the malonic ester method.

THYROXINE

$$HO \diagdown \diagup -O- \diagdown \diagup CH_2-CH(NH_2)-COOH$$

Although the condition recognized as goitre has long been known, it was not until about 1883 that the relationship of this

disease to the activity of the thyroid gland was shown by Kocher (347). With the demonstration by Murray (349) in 1891 of the value of thyroid extract in the treatment of hypothyroidism, interest in the isolation of the active constituent of the gland was aroused, and further impetus was given when Roos (353), working in Baumann's laboratory in Freiburg, showed that the administration of desiccated thyroid gland led to an increase in nitrogen metabolism. Baumann was himself actively engaged in work on the thyroid problem. He was of the opinion that the gland contained a principle of the nature of an enzyme or of a protein and his early attempts at concentration of this principle were directed towards the investigation of its stability.

Roos had investigated the stability of the active substance towards acid. The glands were boiled with 10 per cent sulfuric acid for a day; the insoluble material was then removed and thoroughly extracted with diluted alcohol. This extract contained the active substance. After evaporation of the solution the residue was dissolved in dilute alkali and reprecipitated by acid. This product received the name *thyroiodin*.

Baumann (330) fused the material with sodium hydroxide and potassium nitrate, dissolved the melt in water and acidified with nitric acid. He noted that the fluid was yellow in color and on shaking with chloroform, a violet color, indicative of iodine, passed into the chloroform. Baumann's reaction to this discovery is best expressed in his own words, "Als ich diese Beobachtung zuerst machte, glaubte ich an alles Andere eher, als dass das Jod meiner Substanz angehöre. Indessen blieb ich darüber doch nicht lange im Zweifel, denn alle Reagentien erwiesen sich als völlig rein und frei von Jod." Baumann recognized that the iodine was not in the form of inorganic iodine; he thought that it was combined as a complex in a manner analogous to the form in which iron is contained in hemoglobin. This is clearly brought out in the statement, "Es handelt sich dabei offenbar nicht um eine Wirkung des freien Jods oder eines Jodsalzes, sondern um die Bildung derjenigen specifischen organischen Jodverbindung, welche wir in dem Thyrojodin soweit als möglich isolirt haben. Dieser Vorgang scheint ganz ähnlich demjenigen der Aufnahme

des Eisens zu sein, dessen Wirkung dem Organismus auch erst dann zu Statten kommt, wenn es in diejenige organische Eisenverbindung, aus welcher der Blutfarbstoff besteht, übergeführt ist."

Even prior to the work of Baumann, Kocher (348) had reasoned that certain analogies exist between the therapeutic action of iodine and the active principle of the thyroid gland. "Die Analogie der giftigen Wirkung von Jod und Schilddrüsensaft ist auch darin analog, dass in solchen Fällen ausser den nervösen Symptomen eine ganz bedeutende und rasche Abmagerung eintreten kann, die zum Aussetzen des Mittels nöthigt." At his suggestion Tschirch looked for iodine in the thyroid gland, but reported negative results; it was probably lost during the process of dry ashing.

Baumann's observation at once drew attention to the importance of iodine in animal physiology. Although the nature of the organic compound of iodine that occurs in the gland has only recently been established, it early became clear that the substance was in some way associated with the proteins of the tissue. Oswald (351), in 1900, prepared an active globulin-like material from a water extract of thyroid tissue; this received the name *Jodthyreoglobulin* and appeared to be a mixture of iodine-containing and iodine-free proteins.

Investigation of the physiological properties of iodogorgoic acid by Strouse and Voegtlin (355) showed that this substance did not possess the same activity as desiccated thyroid gland. Hofmeister (341), Oswald (352), Roos (354), and others prepared and studied the properties of iodinated proteins. None of these products had physiological properties like those of Baumann's "Thyroiodin" and it became evident that the iodine-containing complex of the globulin from the thyroid gland was a substance of a highly special nature.

Nürenberg (350) thoroughly investigated the "Jodthyreoglobulin" of Oswald and placed its protein nature beyond doubt; his attempt to isolate iodogorgoic acid from it failed, however, although he succeeded in demonstrating the presence of an organic iodine compound that could be precipitated by silver nitrate in

faintly acid solution. He observed that a commercial product from thyroid glands called "iodothyrine" gave a positive xanthoproteic reaction but negative Adamkiewicz, Millon, and Ehrlich tests. After heating the material in an autoclave a positive Millon reaction was obtained, and after reduction with sodium in alcohol solution, positive tests for tryptophane were secured. This led to the conclusion that iodine is combined with tyrosine and possibly also with tryptophane, in the physiologically active material and the view that one or both of these amino acids are in some way associated with this activity prevailed until the final proof of the structure of thyroxine was obtained.

Nürenberg subjected "Jodthyreoglobulin" to hydrolysis by barium hydroxide; at the end of 30 hours the solution was filtered from an insoluble residue which he considered to be barium carbonate and did not examine. There is little doubt that, had he done so, a clue to the preparation of thyroxine might have been obtained some years before Kendall's work was begun.

The isolation of the substance to which the therapeutic activity of thyroid gland tissue is due was first accomplished by Kendall in 1915 (343). It is unnecessary to describe the preliminary experimental work which led to the isolation; the problem appeared, at the outset, to be relatively simple but proved very difficult in practice. Thyroid tissue contains a physiologically active substance associated with and proportional to its iodine content, and also associated with the globulin fraction. Under certain conditions hydrolysis of this protein could be effected without serious loss of activity, although prolonged enzymatic or acid hydrolysis destroyed it. The active iodine compound appeared to be related in some way to tyrosine or to tryptophane although attempts to isolate the only known natural iodine compound of tyrosine—iodogorgoic acid—from thyroid glands had failed. Furthermore, synthetic substances such as tetraiodohistidine, triiodoimidazole, iodotryptophane, or even iodogorgoic acid itself or iodized proteins did not possess physiological properties in any way analogous to those of the thyroid gland (333). So much had been learned by previous workers.

. Kendall found that severe hydrolysis was necessary to liberate

the active substance and, in view of the instability of the organic
iodine complex in acids, he employed alkaline hydrolyzing rea-
gents.   It was found that 24 hour hydrolysis by 5 per cent sodium
hydroxide gave a solution from which a precipitate separated, on
acidification, that contained about one-quarter of the iodine and
all of the physiological activity.   This material was heated, with
a mixture of sodium and barium hydroxides, for 18 hours at 100°.
An insoluble, iodine-containing precipitate was removed and,
when the solution was neutralized, a second precipitate that
contained a relatively high proportion of iodine separated.   The
material was treated three times successively in the same way,
the proportion of iodine in the acid precipitate rising finally to 47
per cent.   The product was dissolved in alcohol and evaporated
on the water bath; inadvertently it was allowed to go to dryness
and was heated for about an hour.   The addition of alcohol dis-
solved a part of the material and left a white insoluble residue
that weighed 18.6 milligrams; this contained 60 per cent of iodine.
When this was dissolved in sodium hydroxide and the solution
was neutralized and boiled, the substance separated in crystalline
form.   Approximately 200 milligrams of the substance were then
prepared in the same way, and this was soon identified as the
physiologically active principle of the gland; it was named thyrox-
ine (344).

It was two years before Kendall secured his next specimen.
An attempt was made to work with large quantities, but the proc-
ess that went relatively smoothly in glass apparatus on the
laboratory scale was valueless when metal vessels were substi-
tuted.   Finally, after enameled ware or nickel apparatus had
been introduced, thyroxine was again secured and, by 1919,
about 33 grams had been prepared from over 3 tons of fresh
thyroid gland.

The analysis of a substance that contains 65.3 per cent of iodine
is a difficult problem, and Kendall was misled, by a nitrogen deter-
mination that was slightly too high, into drawing the conclusion
that the substance contained three atoms of iodine to one of nitro-
gen.   He formulated the substance as $C_{11}H_{10}NO_3I_3$, and sug-
gested that its structure was probably 4,5,6-trihydro-4,5,6-

triiodo-2-oxy-β-indolepropionic acid. Numerous compounds of thyroxine were prepared the analyses of which did not disagree too seriously with the requirements of this formula, and many of the peculiar properties of thyroxine were explained in terms of hypothetical structural changes.

The chief qualitative evidence brought forward by Kendall in favor of an indole nucleus as the foundation of the structure of thyroxine was the result of a pine-splinter test. Clinical investigation had shown that the substance was undoubtedly the chief active principle of the gland (331) and interest in its fundamental structure was therefore intense. The work of Hicks (340) on the ultraviolet absorption spectra of thyroxine, tryptophane and 2-hydroxyindole-3-propionic acid supported Kendall's views.

Kendall's formulations were, however, by no means generally accepted. The great difficulty of the investigation doubtless prevented many from undertaking to check his results; nevertheless Harington in 1926 published results that showed Kendall's fundamental assumptions of the structure of thyroxine to be incorrect (334). Harington pointed out that the pine-splinter reaction, as employed by Kendall, was not specific for the indole nucleus; furthermore, that the reduced indole structure that had been suggested was inherently improbable on purely chemical grounds. He wrote, "It is fully apparent, therefore, that without considerable further chemical evidence it is impossible to accept the formula proposed by Kendall and that the constitution of thyroxine must be regarded as not proven."

Harington first devised an improved method for the isolation of thyroxine whereby a larger yield of the product might be secured. He employed 10 per cent barium hydroxide for the preliminary hydrolysis of the tissue and the insoluble precipitate that remained, after neutralizing the preliminary hydrolysate, was further hydrolyzed by stronger barium hydroxide. The precipitate obtained by neutralizing this hydrolysate was dissolved in hot dilute sodium hydroxide, barium was removed by sodium sulfate and the filtrate from the barium sulfate was acidified and boiled until the precipitate that separated became granular. This material was dissolved in alkali, alcohol was added, and the

solution was acidified with acetic acid; thyroxine separated in crystalline form in a yield of 0.08 per cent of the dry gland tissue and was further purified by repetition of the final operations. Analysis of this substance, and also of a preparation obtained commercially by Kendall's original method, showed that the ratio of iodine to nitrogen atoms is 4:1 and that the empirical formula should be $C_{15}H_{11}O_4NI_4$. The theoretical requirements of this formula differ very little from those of Kendall's formula except in the case of nitrogen.

Harington next found (335) that the iodine could be quantitatively removed from thyroxine by hydrogen in the presence of a palladium catalyst. The product of this reaction, desiodothyroxine (later called thyronine), had the empirical formula $C_{15}H_{15}O_4N$. It responded positively to Millon's reaction, and gave a ninhydrin reaction; all of its nitrogen was in the amino form and the constitution was therefore that of an α-amino acid in which one oxygen was present as a phenolic group. The proportions of carbon and hydrogen suggested the presence of two benzene rings. Fusion with potassium hydroxide at 250° yielded p-hydroxybenzoic acid, a substance $C_{13}H_{12}O_2$, and a minute amount of quinol; fusion at 310° in an atmosphere of hydrogen gave p-hydroxybenzoic acid and quinol in good yields, together with ammonia and oxalic acid. The substance $C_{13}H_{12}O_2$ possessed one phenolic group; the other oxygen atom was inert. "These experiments in the first place reinforce the suggestion of the presence of two benzene rings, one at least of which has a phenolic or phenol ether group in the *para* position to a side chain from which a two carbon fragment is split off as oxalic acid."

Exhaustive methylation yielded a betaine which, when boiled with alkali, lost trimethylamine and produced an unsaturated acid $C_{16}H_{14}O_4$ that contained one methoxyl. Oxidation of this gave oxalic acid and a neutral substance $C_{14}H_{12}O_3$ which, in turn, yielded a semicarbazone and a phenylhydrazone. This was at first thought to be a ketone, but the action of phosphorus pentachloride on its oxime gave a nitrile instead of an anilide; the oxidation product was therefore an aldehyde and further oxidation of this gave the acid $C_{14}H_{12}O_4$.

"Reviewing the results up to the present point then, we have

in the first place by the potash fusion demonstrated the probable presence in desiodothyroxine of two benzene rings.   The behavior of the compound on exhaustive methylation proves almost with certainty that it is an amino acid; moreover, the presence of one methoxyl group in the unsaturated acid $C_{16}H_{14}O_4$ proves the presence of one phenolic group only in desiodothyroxine; finally the splitting off of oxalic acid by oxidation, with the formation of a residual stable acid is evidence of the presence of a three carbon side chain; on the experiments hitherto described the degradation may be represented thus:

$$HO \cdot C_{12}H_8O \cdot CH_2 \cdot CHNH_2 \cdot COOH \longrightarrow CH_3O \cdot C_{12}H_8O \cdot CH\!=\!CHCOOH$$

$$\longrightarrow CH_3O \cdot C_{12}H_8O \cdot CHO \longrightarrow CH_3O \cdot C_{12}H_8O \cdot COOH$$

The remaining question is therefore the character of the two benzene ring group —$C_{12}H_8O$—.   The two benzene rings composing this group cannot be linked through a carbon atom, since such a linkage would have led, on the above scheme of degradation, to the formation of a ketone (benzophenone derivative) on oxidation in place of the aldehyde actually obtained.   They must therefore be linked either directly (diphenyl) or through the remaining oxygen atom which is as yet unaccounted for.   This indifferent oxygen atom would be difficult to account for on the diphenyl hypothesis, so that the existence of an oxide linkage between the two benzene rings seemed the most probable supposition.   At this point, therefore, it was decided to attempt to meet the degradation by synthesis, proceeding on the hypothesis that the group —$C_{12}H_8O$— represented diphenyl ether minus two hydrogen atoms."

p-Bromoanisol was therefore condensed with the potassium salt of *p*-cresol to yield the compound,

(I)

When boiled with hydriodic acid, this gave

(II)

which was identical with the product of potash fusion of desiodo-thyroxine, $C_{13}H_{12}O_2$. Further, on oxidizing I with permanganate, the acid

$$CH_3O\text{—}\underset{}{\bigcirc}\text{—}O\text{—}\underset{}{\bigcirc}\text{—}COOH$$

(III)

was formed, and this was identical with the acid $C_{14}H_{12}O_3$ produced by the oxidation of desiodothyroxine.

Desiodothyroxine must therefore have the formula,

$$HO\text{—}\underset{}{\bigcirc}\text{—}O\text{—}\underset{}{\bigcirc}\text{—}CH_2\text{—}CHNH_2\text{—}COOH$$

(IV)

The synthesis of this substance was next attempted. p-Bromo-anisol was condensed with potassium phenate and the product was converted to an aldehyde by Gattermann's hydrocyanic acid method.

$$CH_3O\text{—}\underset{}{\bigcirc}\text{—}Br + HO\text{—}\underset{}{\bigcirc} \longrightarrow CH_3O\text{—}\underset{}{\bigcirc}\text{—}O\text{—}\underset{}{\bigcirc}$$

(V)

$$CH_3O\text{—}\underset{}{\bigcirc}\text{—}O\text{—}\underset{}{\bigcirc} \longrightarrow CH_3O\text{—}\underset{}{\bigcirc}\text{—}O\text{—}\underset{}{\bigcirc}\text{—}CHO$$

(VI)

This was identical with the aldehyde $C_{14}H_{12}O_3$ produced during the degradation and was further identified by oxidation to III. The unsaturated acid,

$$CH_3O\text{—}\underset{}{\bigcirc}\text{—}O\text{—}\underset{}{\bigcirc}\text{—}CH{=}CH\text{—}COOH,$$

(VII)

was also prepared by Perkins' method, and was found to be identical with the unsaturated acid $C_{16}H_{14}O_4$ obtained during the degradation.

Desiodothyroxine (IV) was prepared from this aldehyde by two methods; the diketopiperazine condensation method of Sasaki, and the hydantoin condensation method of Wheeler and Hoffmann; in both cases the product was identical with that secured from natural thyroxine.

Thyroxine itself must therefore be a tetraiodo substitution product of this nucleus; the most probable positions for these iodine atoms, were $3,5,3',5'$; because of the analogy with iodogorgoic acid. That this was the case was shown soon afterwards by Harington and Barger (337). On direct treatment of desiodothyroxine with iodine, only two iodine atoms could be introduced. It was therefore evident that the two iodine atoms in the $3,5$ positions must be already in place before the phenyl ether synthesis is effected. The brilliant observation was made that the iodine atom in the 4 position in $3,4,5$-triiodonitrobenzene is preferentially activated in such a way that condensation at this position can be effected with phenols. Quinol monomethyl ether was therefore condensed with $3,4,5$-triiodonitrobenzene,

(VIII)

the nitro derivative was reduced to the amino compound, and this was converted to the nitrile. Hydrolysis then yielded the acid IX.

(IX)

This compound was iodinated to the substance X which, on methylation, yielded XI,

(X)   (XI)

a compound that was identical with the product secured from natural thyroxine that had been subjected to exhaustive methylation followed by oxidation of the side chain. This series of ex-

periments proved that the constitution of thyroxine is represented by formula XII,

$$HO-\underset{I}{\overset{I}{\bigcirc}}-O-\underset{I}{\overset{I}{\bigcirc}}-CH_2-CHNH_2-COOH.$$

(XII)

Thyroxine

The actual synthesis of thyroxine was carried out starting with the nitrile which was converted to the aldehyde XIII.

$$CH_3O-\bigcirc-O-\underset{I}{\overset{I}{\bigcirc}}CN \longrightarrow CH_3O-\bigcirc-O-\underset{I}{\overset{I}{\bigcirc}}CHO$$

(XIII)

It was obvious that the steps that had led to the amino acid desiodothyroxine could hardly be expected to succeed in this case since alkaline reduction would remove the iodine atoms. Success was obtained however when the aldehyde was condensed with hippuric acid and the azlactone produced was reduced by hydriodic acid and red phosphorus.

$$CH_3O-\bigcirc-O-\underset{I}{\overset{I}{\bigcirc}}CHO$$

$$\longrightarrow CH_3O-\bigcirc-O-\underset{I}{\overset{I}{\bigcirc}}CH=C-CO$$
$$\qquad\qquad\qquad\qquad\qquad N\quad O$$
$$\qquad\qquad\qquad\qquad\qquad\searrow\swarrow$$
$$\qquad\qquad\qquad\qquad\qquad C$$
$$\qquad\qquad\qquad\qquad\qquad C_6H_5$$

$$\longrightarrow CH_3O-\bigcirc-O-\underset{I}{\overset{I}{\bigcirc}}CH=C-COOEt$$
$$\qquad\qquad\qquad\qquad\qquad\qquad NHCOC_6H_5$$

$$\longrightarrow HO-\bigcirc-O-\underset{I}{\overset{I}{\bigcirc}}-CH_2-CHNH_2-COOH$$

(XIV)

The product XIV, when treated with iodine in ammoniacal solution, yielded thyroxine, XII, identical in all respects with the substance obtained from the thyroid gland.

It is one of the curious ways of science that two individuals should independently and almost simultaneously arrive at the same conclusions. In a footnote to their article, Harington and Barger state that Dakin had come substantially to the same conclusions as Harington regarding the constitution of thyroxine. On hearing that Harington had communicated a paper, Dakin generously withdrew his paper on the same subject from publication.

Thyroxine, as prepared from the thyroid gland, or by synthesis, is optically inactive owing to racemization during the alkaline hydrolysis by which it is liberated from the gland proteins. Harington therefore attempted the resolution (336) in order to see if the physiological activity of the two isomers is different. Owing to the insolubility of thyroxine and its salts the separation by means of alkaloids was not feasible. He therefore started with the formyl derivative of 3,5-diiodothyronine (the name thyronine was suggested to replace the name desiodothyroxine) and prepared the salts with the two isomeric α-phenylethylamines. An insoluble salt separated from the solution, but this could not be obtained optically pure. The mother liquor, however, yielded a soluble fraction that could be purified by recrystallization. The free acid was recovered from the salt, hydrolyzed and iodinated to thyroxine. l-Thyroxine was obtained from the l-α-phenylethylamine salt and had a specific rotation of $-3.2°$. The isomer was obtained from the d-α-phenylethylamine salt and had a specific rotation of $+2.97$. Physiological tests of these products indicated that the preparation of l-thyroxine was about three times as active as d-thyroxine. If it is assumed that the dextro isomer is physiologically inactive, it would indicate that the resolution had yielded preparations that were respectively 75 per cent pure. It was clear, however, that l-thyroxine is definitely much more active physiologically than its isomer, and this is therefore probably the isomer that occurs in nature.

Ashley and Harington (329) in 1928 reported the preparation of a series of peptides containing thyroxine together with esters and other derivatives of thyroxine. A year later Harington and Randall (338) and Foster (332) almost simultaneously announced the isolation of diiodotyrosine from the thyroid gland. Some indications that such a compound might be present had already been given by Ingvaldsen and Cameron (342). This demonstration of the presence of diiodotyrosine in the thyroid gland add support to the view expressed by Harington and Barger that thyroxine is probably derived from tyrosine through the intermediate stage of diiodotyrosine, two molecules of which may undergo oxidative coupling with the loss of one side chain.

A year after the appearance of Harington's first paper on thyroxine, Kendall (345) announced that the greater yield of thyroxine which Harington had reported by the use of barium hydroxide instead of sodium hydroxide for hydrolysis could not be attributed to the hydrolyzing agent but rather to the fact that English thyroid glands contain a greater percentage of thyroxine. Kendall explained the discrepancy between the molecular weight he had found and that reported by Harington as possibly due to the addition of some substance during the preparation of his thyroxine derivatives. Kendall's molecular weight was based on the iodine content of the acetyl derivative, the ureide, and the sulfate of thyroxine. Kendall accepted Harington's proof for the constitution of thyroxine in the following words: "I congratulate Harington in bringing to a successful close the identification and synthesis of one of the most interesting substances known."

In a subsequent paper Kendall and Simonsen (346) showed, in confirmation of previous work by others, that there is a seasonal variation in the iodine content of the thyroid glands of American animals which may amount to 300 per cent.

In 1930 Harington and Salter (339) succeeded in preparing thyroxine from crude thyreoglobulin and from gland tissue that had been hydrolyzed by the successive action of pepsin and trypsin. The product was obtained in small yield but was identical with synthetic optically active thyroxine.

OXYGLUTAMIC ACID

$$CH(OH)—CH(NH_2)—COOH$$
$$|$$
$$CH_2—COOH$$

In 1908 Osborne, Leavenworth and Brautlecht (363) investigated the relationship between the quantities of ammonia that could be obtained from proteins by hydrolysis and the quantities of aspartic and glutamic acid yielded by the same proteins. Hlasiwetz and Habermann (361) had suggested, as early as 1873, that these two acids were probably combined in the protein in the form of amides, and Osborne and his associates attempted to obtain quantitative evidence that this was the case. They calculated the quantity of ammonia that might be expected on this assumption and compared the theoretical with the actual amount. Striking agreement was found in many cases. "Marked exceptions, however, are shown by the proteins of the cereals, for which the amount calculated falls very much below that found analytically." They pointed out that small deficits might be due to "the uncertainties attending the isolation of these dibasic acids, but in the case of the cereal proteins these differences are so large that it does not seem possible to explain them in this way. . . . . It is therefore probable that the cereal proteins in some way differ in structure from all the others which have been examined, and that they may possibly contain some other dibasic acid not yet isolated from their decomposition products."

This prediction was fulfilled ten years later by the discovery of β-hydroxyglutamic acid by Dakin as a result of the application of a new method and of the reintroduction of an old and completely forgotten method, into the technique of amino acid analysis.

It has already been pointed out that Ritthausen's discovery of aspartic acid among the products of hydrolysis of proteins was due to his observation that the mother liquor from the crystallization of the glutamic acid contained a strongly acid substance. He had therefore neutralized it with barium carbonate and added alcohol; the barium salt of the acid thereupon separated. Later he employed the calcium salt, and similar methods were used by Schulze (364) for the separation of glutamic and aspartic acids

from protein hydrolysates.   The possibilities of this method seem, however, to have been overlooked by all save one subsequent investigator[18] until 1914, when Foreman (360) became interested in the problem of improving the methods for the estimation of the dicarboxylic amino acids.   He wrote:

"It seemed possible that a method might be based on some essential difference between the two types—dibasic and monobasic amino acids. As it is well known that the calcium salts of non-nitrogenous dibasic organic acids have a much higher degree of insolubility in water or alcohol than the calcium salts of non-nitrogenous monobasic acids, it was thought that the same principle might apply in the case of the dibasic and monobasic amino acids.   Experiments were therefore made with a view to testing this matter.

"Calcium chloride solution was added to a solution of the amino acids obtained by the hydrolysis of caseinogen.   No precipitate resulted, and on adding much alcohol only a small precipitate was obtained. When, however, another portion of the same solution was made alkaline with lime, a copious precipitate appeared on the addition of alcohol. The precipitate seemed to increase somewhat in quantity when the free ammonia was removed before the alcohol was added.

"Solutions of all the monoamino acids found in proteins, with the exception of oxyproline, were then treated separately with lime and alcohol.   Glutaminic and aspartic acids, cystine and tyrosine all gave calcium salts insoluble in alcohol.   The calcium salts of the other monoamino acids, however, were found to be very soluble in alcohol, and the solutions all remained perfectly clear when the alcohol was added. Pyrrolidonecarboxylic acid has since been tried in the same way, and a copious precipitate was obtained.

"Since these observations were made I have found that Abderhalden and Kautzsch (356) have made the calcium salts of aspartic, glutaminic,

[18] C. T. Mörner in 1913 (Zur Charakteristik des 3,5-Dibromtyrosins.   Z. physiol. Chem. 88, 124–37 (1913)) observed that dibromotyrosine, tyrosine, aspartic acid, and glutamic acid all yield precipitates when their solutions; in an excess of aqueous barium hydroxide, are treated with five volumes of alcohol.   A number of other amino acids were tested but did not give precipitates.   He made use of this property for the isolation of dibromotyrosine (see p. 165) from an hydrolysate of the horn-like skeleton of *Primnoa lepadifera*, and states that tyrosine, glutamic acid, and aspartic acid together with oxalic acid were also isolated in pure form from the fraction secured by precipitation with barium hydroxide and alcohol; no details of this work are given however.

and pyrrolidonecarboxylic acids separately from the pure substances in a similar way. They have not suggested, however, that any quantitative use could be made of these facts in reference to separations from hydrolytic products derived from proteins."

Foreman had obviously overlooked the early papers of Ritthausen and of Schulze, as well as Mörner's more recent work.

In working up the material precipitated from a casein hydrolysate by calcium hydroxide and alcohol, Foreman obtained the amino acids in dry form and extracted the mixture with glacial acetic acid. The extract, on evaporation, yielded a gum that was found to contain considerable pyrrolidonecarboxylic acid doubtless derived by the internal condensation of glutamic acid during the evaporations. Not all of the gum could be so accounted for and indirect evidence was secured that a substance soluble in glacial acetic acid was present which contained about 10 per cent of amino nitrogen. "The identity of this constituent of the gum has not yet been established." Foreman's investigations were interrupted by the outbreak of the war.

Dakin, in 1918 (357), reported the results of an extensive investigation of the methods of separation of the amino acids. He described the now well-known butyl alcohol method for the extraction of the monoamino acids and the isolation, from the aqueous solution that contained the non-extractable basic and dicarboxylic amino acids, of a new substance, β-hydroxyglutamic acid. This solution was an exceptionally favorable starting point for the investigation of the dicarboxylic acids as was shown by the results for the glutamic acid determination. Dakin obtained 21.6 per cent of this acid by direct crystallization of the hydrochloride from a solution derived from casein, a result much higher than those of previous investigators with the exception of Foreman who had obtained 21.8 per cent. The mother liquor from the glutamic acid hydrochloride was treated with calcium hydroxide and alcohol, as described by Foreman, and the aspartic acid was removed from the precipitated material as its lead salt. A small amount of basic substance was then removed with phosphotungstic acid. After freeing the solution from reagents it was found still to contain a strong organic acid. This was therefore

precipitated as its silver salt by the alternate addition of silver nitrate and sodium hydroxide. The silver compound was decomposed by hydrogen sulfide and the solution was concentrated at low temperature to a sirup. Thick prisms began to separate when this had stood in a vacuum desiccator for some time.

The new acid was optically active. It was extremely soluble in water and gave salts with metals that did not crystallize readily. It titrated as a monobasic acid in water, as a dibasic acid in the presence of formaldehyde. Its analysis and molecular weight corresponded to the formula $C_5H_9O_5N$, and its nitrogen, provided the preparation had not been heated unduly, was all amino nitrogen. Heated above 100° it lost water and the amino nitrogen was converted into imino nitrogen, a reaction clearly analogous to the formation of pyrrolidonecarboxylic acid from glutamic acid. A pyrrole reaction could be secured after heating the product with zinc dust. Glutamic acid was produced by reduction of the new acid with hydriodic acid. The acid did not give a lactone; substitution of an hydroxyl in the γ-position was therefore improbable. Oxidation with chloramine-T yielded an aldehyde which, in turn, gave an osazone of the composition $C_3H_5COOH(N \cdot NH \cdot C_6H_4NO_2)_2$ with p-nitrophenylhydrazine. The formation of this product indicated the presence of an hydroxyl group on the carbon atom adjacent to the α-amino group. Dakin wrote, "the above results can hardly be reconciled with any other structure for the acid than that of an α-amino-β-hydroxyglutaric acid, i.e. β-hydroxyglutamic acid, $COOH \cdot CH(NH_2) \cdot CH(OH) \cdot CH_2 \cdot COOH$." The accuracy of this formula was attested by the preparation and analysis of salts of silver, copper, lead, calcium, and barium and of the naphthalenesulfonyl chloride derivative. Furthermore a number of characteristic color reactions with phenols were described.

The presence of this acid among the products of hydrolysis of casein recalled the statement of Skraup (365) that hydroxyaspartic acid was one of the products of the hydrolysis of casein. "A search for tartaric and racemic acid among the products of the action of nitrous acid upon the dicarboxylic acids of caseinogen gave negative results, thus indicating the probable absence of hydroxyaspartic acid."

Dakin pointed out the relationship of the new acid to the material described by Foreman. He wrote, "While the writer's experiments originated from a totally different direction and were not influenced by Foreman's observations, there is little doubt that if the latter had been able to pursue his investigations uninterruptedly he would have isolated β-hydroxyglutamic acid."

A later paper by Dakin (358) described a synthesis of oxyglutamic acid. This proved to be extremely difficult but was finally accomplished; glutamic acid furnished the starting material. The position of the hydroxyl group was established by a synthesis of the osazone that had been obtained from the product of the oxidation of oxyglutamic acid with chloramine-T, and useful salts with various alkaloids were described. The new acid was also isolated from gliadin and glutenin. Dakin subsequently (359) obtained 2.5 per cent of it from zein and Jones and Wilson (362) have found as much as 7.7 per cent of it in gliadin. These observations are therefore verifications of the prediction of Osborne in 1908.

METHIONINE

$$CH_2-CH(NH_2)-COOH$$
$$|$$
$$CH_2-S-CH_3$$

In 1921 Mueller (367) published a short paper in which he recorded the observation that there was a substance present in meat infusion, as well as in hydrolysates of some proteins, that was essential for the growth of hemolytic streptococcus and of certain strains of pneumococcus. This substance could be removed from beef infusion by boiling with Norit for 15 minutes. The cocci would grow, however, if to such a culture medium 1 per cent of commercial peptone were added, although a 1 per cent peptone solution by itself, with suitable salts and sugars added, did not furnish an adequate medium for the streptococcus. A solution containing the products of sulfuric acid hydrolysis of casein was an equally effective adjuvant, and preparations were secured from hydrolyzed edestin or meat protein that were also valuable, although one prepared from egg white was only weakly active.

Hydrolysates of wheat gluten, gelatin, wool, and silk were ineffective. These observations indicated that the active substance was probably an amino acid of limited distribution, and published analyses of these proteins indicated that none of the known amino acids possessed a distribution similar to that of the hypothetical active substance.

Mueller therefore attempted a fractionation of casein hydrolysates by various reagents and found that mercuric sulfate precipitated the active substance in the presence of 5 per cent sulfuric acid. This led to tests upon cystine, tyrosine, tryptophane, and histidine, the amino acids known to be precipitated by this reagent, but all were inactive. Further tests indicated that the substance was not precipitated by silver and alkali in the histidine fraction, but was partly precipitated along with arginine; it was apparently destroyed by phosphotungstic acid.

Later (368) Mueller obtained the impression, from attempts at fractional precipitation by mercuric sulfate, that two factors might be present, both of which were necessary for the growth of the streptococcus. Further work showed that the filtrate from a silver precipitation at "moderately alkaline reaction to litmus" gave a precipitate with mercuric sulfate that was inactive, and a filtrate from which tyrosine separated on evaporation, after removal of reagents. The mother liquor then deposited nodular masses of needles which were quite soluble in water and in 70–80 per cent alcohol. These were obviously impure but were so active that 0.01 milligram added to 25 cubic centimeters of medium sufficed for the growth of the streptococcus. The material gave a moderate reaction with Folin's phenol reagent but no nitroprusside test. It contained 10.6 per cent of nitrogen and sulfur was also present although no lead-blackening sulfur test could be obtained. Cystine was therefore absent and the high proportion of nitrogen showed that little tyrosine was present in the product.

In 1922 (369) Mueller announced the discovery of a new sulfur-containing amino acid among the products of hydrolysis of casein. It is a curious fact that Mueller was led to this discovery by a biological test, namely an acceleration of the growth of streptococcus; when the substance was finally obtained in a

nearly pure form, however, it had no particular influence on the growth of the bacteria. After the discovery of the non-cystine sulfur his investigation had been continued purely from the chemical point of view. The procedure for the isolation of the new substance was laborious. The protein was hydrolyzed by sulfuric acid, neutralized by sodium carbonate and the solution was treated with mercuric sulfate. The precipitate was decomposed by hydrogen sulfide and the solution was treated again with mercuric sulfate. This removed much extraneous matter and the sulfur compound remained in the filtrate. More impurities were removed by a silver precipitation at alkaline reaction and the sulfur compound, in a yield of 10 grams from 30 pounds of casein, was secured by fractional crystallization from the filtrate. Analysis led to the formula $C_{11}H_{23}SN_2O_4$, but later work demonstrated that the preparation contained phenylalanine as an impurity. Mueller showed, however, that the nitrogen was all in the amino form, that the substance was a neutral amino acid and that the sulfur was extraordinarily stable.

In 1923 Mueller repeated the work (370) on a large scale and improved the method of preparation materially. The procedure now consisted of hydrolysis followed by neutralization of the hydrolysate and precipitation of the product by mercuric sulfate at a neutral reaction to litmus. The precipitate was thoroughly washed and then extracted by hot 2 per cent barium hydroxide solution four successive times. The extracts were freed from mercury by barium sulfide and then from barium. After concentration, mercuric chloride was added and the precipitate was removed, washed, and decomposed with barium sulfide. Reagents were removed from the filtrate which was then evaporated *in vacuo*. The crystals that separated were redissolved by heating and several volumes of alcohol were added; the product then separated in well-formed crystals. The material was, however, still impure. Further purification was effected, although with considerable loss, by a repetition of the precipitation with mercuric chloride. The yield varied from 0.2–0.4 per cent of the casein.

. Analysis of this material and determination of the molecular

weight led to the formula $C_5H_{11}SNO_2$. It melted at 280–281° in a sealed tube and had $[\alpha]_D^{20} = -7.2°$. A copper salt and naphthylisocyanate derivative were prepared and analyzed. Mueller prepared the same substance after an alkali hydrolysis of casein and also after sulfuric acid hydrolysis in a procedure in which hydrogen selenide was employed for the decomposition of the mercury compounds in order to remove the possibility that sulfur had been introduced artificially. He likewise got it from egg albumin, edestin, and wool. A possible structure C_2H_5—S—CH_2—$CH(NH_2)COOH$, was eliminated by synthesis. Although many properties of the new substance corresponded with those of ethyl cysteine, the stability of the sulfur in the two compounds was different. When ethyl cysteine was treated with boiling dilute alkali ethyl mercaptan was split off; the new substance withstood this treatment. A biological test demonstrated (371) that the new amino acid was oxidized in the animal body.

In 1925 Odake (372) isolated from yeast extracts a substance that possessed the properties of Mueller's new amino acid. Barger and Coyne (366) in 1928 became involved in a study of the non-cystine sulfur of proteins and prepared Mueller's substance. Inasmuch as Mueller had eliminated ethyl cysteine as a possibility and the methylthiol group is, like methoxyl, fairly common in nature, Barger and Coyne felt that the substance probably was a methylthiol derivative of one of the butyric acids. A test showed that Mueller's substance contained one methylthiol group, it was almost certainly an α-amino acid and consequently, its constitution was probably represented by one of the following formulas.

CH₃—CH₂—C(NH₂)—COOH   CH₃—S—CH₂—CH₂—CH(NH₂)COOH
          |
          SCH₃
          (I)   (III)

CH₃—CH—CH(NH₂)—COOH   CH₃—S—CH₂—C(NH₂)—COOH
      | |
      S—CH₃   CH₃
      (II)   (IV)

Of these formula III was the most likely because of the analogy with cheirolin, a mustard oil present in wallflower seeds, which

has the constitution CH_3—SO_2—CH_2—CH_2—CH_2—NCS, and also because phenyl ethyl mustard oil is obviously allied to phenylalanine. "We therefore decided to synthesize the racemic substance (III), and indeed found it to be identical with Mueller's acid, except as regards optical activity. Later, Dr. Mueller informed us that he had himself arrived at the same constitution and had unsuccessfully attempted to prove it by synthesis. Since the amino acid has a good title to be regarded as a constituent of protein, a shorter name than γ-methylthiol-α-amino butyric acid seems desirable, and, after consultation with Dr. Mueller, we suggest for it the name methionine, in allusion to the characteristic grouping."

Barger and Coyne's synthesis started from β-methylthiol-propaldehyde, CH_3—S—CH_2—CH_2CHO, prepared from methyl mercaptan and sodium ethoxide by hydrolysis of the acetal. This product yielded the required amino acid, although in small yield (6 per cent), when the Strecker cyanohydrin method was applied.

A somewhat more satisfactory synthesis is that of Windus and Marvel (373) in which β-chloroethyl alcohol was allowed to react with sodium methyl mercaptide to form methylthiolethyl alcohol. This product was converted to the chloride with thionyl chloride and this served as the starting point of a conventional malonic ester synthesis.

OTHER PRODUCTS OF PROTEIN HYDROLYSIS

In addition to the twenty-one amino acids that have been clearly characterized as definite products of the hydrolysis of proteins there is one unique substance that has hitherto been obtained only by a single investigator from a single source. This is 3,5-dibromotyrosine, which was isolated in 1913 by C. T. Mörner from the horny skeleton of the coral *Primnoa lepadifera* (402). Mörner's identification of the product is so complete in every detail and there is so little chance for confusion with other compounds in the case of a substance that contains 47.2 per cent of bromine, that the exclusion of this interesting substance from the list of well established amino acid products of the hydrolysis of proteins is perhaps arbitrary. Nevertheless it has seemed wise to

adhere to the criteria adopted in the introduction of this paper and await the isolation of dibromotyrosine by some other investigator.

3,5-Dibromotyrosine was first synthesized by v. Gorup-Besanez (398) in 1863 by the action of bromine vapor on dry tyrosine. The identification of iodogorgoic acid from *Gorgonia Cavolinii* as 3,5-diiodotyrosine as a result of the work of Wheeler and Jamieson and of Henze, and the observation that bromine was present, in addition to iodine, in more than 50 different species of marine animals allied to the gorgonian corals, led Mörner, about 20 years ago (401), to search for dibromotyrosine in one of these. The axial skeleton was hydrolyzed by heating with barium hydroxide and, after cooling and removing the crystals of reagent, five volumes of alcohol were added. The precipitate was found to contain 78 per cent of the bromine; it was decomposed by carbon dioxide and the solution was treated with excess of basic lead acetate. After a reprecipitation with this reagent, the solution on evaporation yielded a crude crystalline product rich in bromine. More of the same substance was secured from the filtrates from the lead precipitates and, after extensive recrystallization, a product was finally obtained that was free from tyrosine and corresponded in all respects with synthetic 3,5-dibromo-*dl*-tyrosine. The yield was small, less than 0.2 per cent, and accounted for less than 3 per cent of the bromine of the original material.

Mörner's brilliant observation leaves no doubt whatever that dibromotyrosine, or bromogorgoic acid as he termed it, is a substance of physiological importance in at least one group of organisms. A further study of this substance would be highly desirable.

A method for the synthesis of dibromotyrosine much more convenient than that of v. Gorup-Besanez has been described by Zeynek (415).

Many other substances have been described by various investigators, from time to time, as definite products of the amino acid type derived by hydrolysis from proteins. None of these has been characterized in such a way as to comply with the criteria adopted

in the introduction of this paper; some of them, however, are substances that might logically be expected to be present among the products of hydrolysis of proteins, while others are almost certainly either mixtures of known amino acids or peptide-like compounds of these. Further investigation is required in each case before the substance in question can be safely accepted as a primary constituent of the protein. In the following paragraphs a number of these preparations are discussed, but no attempt has been made to present an exhaustive treatment of them.

In 1904, Skraup (410) described the isolation from casein of a number of amino acids; diaminoglutaric acid, $C_5H_{12}O_4N_2$, diaminoadipic acid, $C_6H_{14}O_4N_2$; oxyaminosuccinic (hydroxyaspartic) acid, $C_4H_7O_5N$; dioxydiaminosuberic acid, $C_8H_{16}N_2O_6$; a tribasic acid, "Caseansäure," $C_9H_{16}N_2O_6$; and a dibasic acid, caseinic acid, $C_{12}H_{16}N_2O_5$.

The amounts of oxyaminosuccinic acid, diaminoadipic acid, and dioxyaminosuberic acid Skraup obtained were very small and the preparations were of questionable purity; the constitution of none of them was established. With the exceptions of caseinic acid and possibly hydroxyaspartic acid, which has received a good deal of attention (386), none of these merits serious consideration at the present time. Skraup's caseinic acid was precipitable by phosphotungstic acid, and was isolated as the copper salt. On decomposing this he obtained two caseinic acids of the same composition but of different melting points. The first was optically active, the second was inactive.

Shortly after Skraup submitted his paper for publication, Fischer and Abderhalden (390) described the isolation of a compound from casein that was, in some respects, similar to Skraup's caseinic acid. Their product had a composition that could be represented by the formula $C_{12}H_{26}N_2O_5$; it was a saturated aliphatic oxyamino acid and received the name diaminotrioxydodecanic acid. This substance was found to occur in impure tyrosine preparations and could be separated by precipitation with phosphotungstic acid. The chemical constitution was not determined. In 1917 Fischer (389) stated that the individuality of this substance had become doubtful and it was therefore omitted from his list of established amino acids.

In 1927 Fränkel and Friedmann obtained (394), from a pancreatic digest of casein, a product that they regarded as possibly identical with Fischer and Abderhalden's substance. No proof of the constitution was presented, however, and the formula they ascribe to the anhydrous substance is so close to twice that of leucine as to suggest that they may have had an impure specimen of racemic leucine in hand.

Abderhalden and Kempe in 1907 (376) obtained, as a product of the hydrolysis of casein, a preparation that was considered to be oxytryptophane. In 1924 (378) Abderhalden and Sickel reëxamined this substance and concluded that it was 6-hydroxy-2,3,dihydroindolyl-3-alanine. Somewhat later (379), however, they prepared more of the material, purified it more thoroughly and arrived at the conclusion that it was a mixture that consisted chiefly of a peptide of tyrosine and proline. The original preparation probably contained tryptophane as well.

The possibility that a third isomeric leucine may occur in proteins has been frequently discussed but the difficulty of demonstrating this is so great that two investigators only have advanced definite claims in this connection. Thudichum, in 1901 (411), described experiments on the products of hydrolysis of neuroplastin. The crude leucine, secured by direct crystallization, was purified as the copper salt and a product was finally obtained that differed from ordinary leucine in solubility. Convincing evidence that this was n-leucine was, however, not provided.

Abderhalden and Weil, in 1912 (380), investigated the proteins of nerve tissue and isolated a substance, of the composition of leucine, which did not agree in optical properties with either leucine or isoleucine. Later (381) they presented evidence in support of the view that this substance was α-amino-n-caproic acid. They at first suggested the name "caprine" for this substance, but Abderhalden, Froehlich and Fuchs (375) substituted the name norleucine. Abderhalden and Weil (382) contrasted the melting points, rotations and other physical properties of their preparation with those of leucine and of isoleucine, and also compared the melting point of the naphthalenesulfonyl chloride derivative with that of synthetic norleucine; the agreement was

fairly close. They further drew attention to the differences in color of the preparations of the three copper salts as evidence of differences in structure.

Unfortunately Abderhalden and Weil give no precise description of the method by which their leucine isomer was secured. They apparently relied on fractional crystallization. In view of the difficulty of separating the two known isomeric leucines by this method, not to mention the difficulty of removing traces of valine, which might have important effects upon the physical properties of the resulting product, it would seem only conservative to require still more convincing evidence of the existence of norleucine among the products of hydrolysis of proteins than that given by Abderhalden and Weil.

Aminobutyric acid was stated to be a product of the alkaline hydrolysis of silk fibroin by Schützenberger and Bourgeois (409), but a reinvestigation by Fischer and Skita (392) showed that this conclusion was incorrect. Fischer and Mouneyrat (391) prepared synthetic aminobutyric acid and resolved it into the optical isomers; the properties of the active substance are therefore on record.

Foreman, in 1913 (393), during an investigation of a proline fraction secured from casein, obtained a preparation the composition of which corresponded to aminobutyric acid. The crude material was extracted with chloroform and then with cold absolute alcohol; the insoluble residue yielded the preparation in question. The evidence for its identity consisted solely in the ultimate analysis of the free acid and of the copper salt. No other derivatives were prepared and the melting point was much lower than that given by Fischer and Mouneyrat. Abderhalden and Weil, in 1913 (382), likewise claimed to have isolated aminobutyric acid from several proteins but provided no experimental evidence in support of this.

Van Slyke and Hiller (413) compared the histidine content of a number of proteins, as indicated by the Koessler and Hanke colorimetric method, with that determined from the non-amino nitrogen in the phosphotungstate precipitate after the removal of arginine. In the cases of casein, edestin, and fibrin the agree-

ment was good, but in the case of gelatin the values obtained from the non-amino nitrogen were much larger than those from the colorimetric method. In attempting to identify the substance responsible for this difference, Van Slyke and Hiller removed lysine, arginine, and histidine from the fraction precipitated by phosphotungstic acid; recrystallization of the material in the filtrate yielded a product in which the ratio of total to amino nitrogen was 2:1. Analysis of the copper salt prepared from this indicated the composition $(C_7H_9O_4N_2)_2Cu$. Van Slyke and Robson (414) later concluded, from the ratio of amino nitrogen to total nitrogen, and from the fact that the product gave a test for the pyrrol group, that the compound may be dihydroxypyrrole-alanine. The structure has not been confirmed by synthesis nor has the free amino acid been prepared in a crystalline state.

Schryver and Buston (405) have reported the presence of hydroxyvaline and hydroxyaminobutyric acid among the soluble barium carbamates derived from the oat protein. This fraction was separated by means of the zinc salts into (a) a sub-fraction only slightly soluble in cold water; this contained leucine; (b) a sub-fraction easily soluble in cold water but insoluble in alcohol; this yielded alanine and valine; (c) a sub-fraction soluble in both cold water and alcohol; the two new amino acids occurred in this fraction. It was found that hydroxyaminobutyric acid yields a copper salt insoluble in methyl alcohol while the copper salt of hydroxyvaline is soluble in methyl alcohol. Analyses of the copper salt, the benzoyl, and the phenylisocyanate derivatives of the amino acid obtained from the copper salt insoluble in methyl alcohol, suggested that this substance was hydroxyamino-butyric acid. The positions of the hydroxyl and amino groups were not established nor was an attempt made to synthesize this amino acid.

Analyses of the copper salt, the benzoyl and the phenylisocyanate derivatives of the amino acid obtained from the copper salt soluble in methyl alcohol, indicated a composition corresponding to that of hydroxyvaline. The positions of the amino and hydroxy groups were not determined.

Gortner and Hoffmann (397) isolated a substance from the pro-

tein teozein, which they consider to be hydroxyaminobutyric acid, and the presence of the same substance in casein is suggested by the work of Rimington (404). Brazier (385) has reported the presence of hydroxyvaline in zein.

Schryver, Buston and Mukherjee (408) found that the glycine fraction, secured during the separation of the products of hydrolysis of fish gelatin by the carbamate method, contained a base that could be separated as its phosphotungstate and reprecipitated as a mercury salt. It was hygroscopic and absorbed carbon dioxide from the air and both the copper salt and the crystalline nitrate of this base were very deliquescent. The base was not precipitated by silver salts from solutions made alkaline with barium hydroxide and, consequently, it could be separated from arginine and histidine. Its composition corresponded to that of hydroxylysine. Schryver and Buston (407) believed that the hydroxy group is in the β-position although no direct evidence from synthesis was offered. Evidence for the presence of this basic amino acid in the proteins of oats, cabbage leaf, hemp seed, and gelatin was obtained.

Schryver and Buston (406) have also described a substance they isolated from oat protein and the protein of the castor bean, the composition of which corresponded to the formula $C_8H_{15}O_3N_3$. They gave it the name protoctine "to indicate a base with eight carbon atoms, derived from proteins." Its constitution was not determined. The product was soluble in water and in absolute alcohol, but insoluble in ether. It was precipitated from solution by mercuric chloride and barium hydroxide but not by silver nitrate and alkali. In alkaline solution it gave an orange-red color with diazobenzenesulfonic acid. The substance contained one amino, one carboxyl, and one hydroxyl group.

Fränkel and Monasterio (395) dialyzed a digest obtained from hemoglobin and treated the concentrated dialysate with methyl alcohol. A fraction was secured which, on analysis, had the composition $C_{22}H_{46}N_4O_7$. The preparation contained four amino groups and three carboxyls. Its structure was not elucidated but a name *Tetratrisäure* was given to it. No evidence that this is a homogeneous substance was presented.

Abderhalden and Bahn (374) have recently described indirect evidence for the presence of α-amino-n-valeric acid or norvaline among the products of hydrolysis of globin, and Abderhalden and Reich (377) have made similar observations upon products derived from casein. Their method is entirely new. The valine-leucine fraction secured by the ester distillation method was subjected to fractional crystallization and a valine fraction was isolated. This was treated with nitrosyl bromide whereby it was converted to α-bromovaleric acid; this was in turn treated with ammonia, and the rate at which bromide ion was split off was determined. This rate was compared with the rates at which bromide is removed from synthetic α-bromo-n-valeric acid and from the analogous compound secured from natural valine, α-bromoisovaleric acid. The curves for the products secured from globin and from casein corresponded with that of α-bromo-n-valeric acid. The conclusion was drawn that norvaline is one of the products of hydrolysis of these two proteins.

This review would be incomplete without a reference to two amino acids that have not yet been isolated from the products of hydrolysis of proteins but which further investigation may be expected to reveal; these are thiolhistidine and dihydroxyphenylalanine.

Evidence for the presence of ergothioneine, the betaine of thiolhistidine, in human and in animal blood has been obtained by Eagles and Johnson (387) and by Newton, Benedict, and Dakin (403). This substance, which was originally discovered in ergot of rye by Tanret, has been synthesized by Barger and Ewins (384). Eagles and Vars (388) investigated the physiology of ergothioneine and found that it was absent from the blood of pigs that had been restricted to a diet in which casein formed the source of protein, but appeared in the blood soon after the same animals were placed upon a diet that contained a large proportion of corn. This observation suggested that ergothioneine arises from a precursor in the corn diet and tests upon zein revealed that this protein, on hydrolysis, yields a solution that responds positively to Hunter's highly specific color test for the thiolimidazole ring. Weakly positive tests were obtained with a number of other pro-

teins but casein and gelatin do not yield the substance responsible for the test. It is probable that the substance that gives this test is thiolhistidine and searches for this substance have been conducted in several laboratories although hitherto without success. Experiments by Eagles and Vars in 1928 in the laboratory of one of the writers showed, however, that the reactive substance can be precipitated by silver salts at a reaction somewhat more acid than that necessary to precipitate histidine, and this observation has also been made by Ashley and Harington (383). The substance rapidly disappeared, however, during the attempts to recover it from the silver precipitate.

l-2-Thiolhistidine has been synthesized by Ashley and Harington.

Dihydroxyphenylalanine was first found by Torquati (412) in 1913, in aqueous extracts of the pods and sprouts of *Vicia faba*, but the identity of the substance was not recognized by him. Guggenheim (399), in the same year, pointed out that Torquati's substance was probably dihydroxyphenylalanine and proved this by repeating the preparation and definitely establishing the constitution and its identity with the synthetic substance prepared by Funk (396). Miller (400) has since identified it in an extract of the velvet bean (*Stizolobium deeringianum*) and has also shown that it is probably widely distributed in this genus.

No attempts to obtain dihydroxyphenylalanine from hydrolysates of proteins have come to our attention but Guggenheim pointed out that owing to the ease with which it is oxidized this substance may be in part responsible for the humin observed when proteins are hydrolyzed by acids. Further investigation will alone decide this.

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THE CALCULATION OF CHEMICAL EQUILIBRIUM FROM SPECTROSCOPIC DATA[1]

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On account of the high accuracy with which spectroscopic data are obtained, thermodynamic quantities calculated from these data are far more reliable than those obtained by direct thermal measurements. It may be predicted that in the future thermodynamic quantities will be calculated from spectroscopic data, whenever they are available. The band spectra of most diatomic molecules have been analyzed completely. Little progress has been made with polyatomic molecules because of their complexity, although there is reason to believe that the Raman spectrum may furnish sufficient data for approximate thermodynamic calculations, at least. The discussion of this article[2] will be limited to monatomic and diatomic molecules. The calculation

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[1] Presented at the Symposium on the Application of the Quantum Theory to Physical Chemistry at the eighty-first meeting of the American Chemical Society at Indianapolis, March 31, 1931.

[2] The symbols used in this article are as follows:

T = absolute temperature.

k = molecular gas constant = 1.37×10^{-16} ergs/degree.

N = total number of molecules.

N_0 = 6.06×10^{23}, the number in one molecule.

R = $N_0 k$ the molal gas constant.

m = the mass of one molecule.

h = Planck's constant = 6.55×10^{-27} ergs/second.

N_n = the number of molecules in the nth energy level.

ϵ_n = the energy of one molecule in the nth energy level.

e = the base of the system of natural logarithms.

I = the moment of inertia of a molecule.

ω = the frequency of vibration of the molecule.

p_n = the multiplicity of the nth level.

i = the quantum number n in a summation.

Σ = sign of summation to be taken over all energy levels from zero to infinity. The limits of the summation need not always be printed.

of heats of dissociation will not be discussed in this paper, since it belongs to the theory of spectroscopy rather than to statistical mechanics.

The ultimate goal in the analysis of the band spectrum of a molecule is the determination of the possible energy states of the molecule. From the conventional method of representing these energy states graphically (figure 1) they have come to be known as energy "levels." In the case of the diatomic molecule these levels are somewhat arbitrarily classified as rotational, vibrational or electronic levels. This classification has the ad-
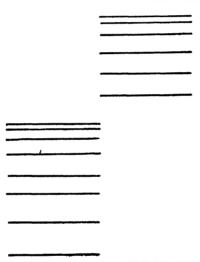
FIG. 1. ENERGY LEVELS OF A MOLECULE

vantage that the formulas for the spacing of the different kinds of levels are given for the simpler cases by the quantum mechanics, and in the more complex cases may be represented by empirical formulas. These formulas all contain a quantum number which, usually, begins with zero and takes on successive integral values.

Both diatomic and monatomic molecules in the gaseous stage possess kinetic energy of translation. This kinetic energy of translation is usually supposed to vary continuously, but in this paper we shall assume that translational energy also is quantized, and we shall proceed later to derive an expression for the trans-

lational energy levels. Consequently the possible energies of any gaseous molecule are given by a set of discrete energy levels, which are often very numerous and very close together, but which never become a continuum. When a molecule has a certain energy we shall say that it lies on a certain energy level.

THE DISTRIBUTION LAW

The Maxwell-Boltzmann distribution law states that N_n, the number of molecules in the nth energy state or level, is given by the expression,

$$N_n = NKe^{-\frac{\epsilon_n}{kT}} \tag{1}$$

where N is the total number of molecules, K is a constant, e is the base of the system of natural logarithms, ϵ_n is the energy of the nth level, k is the molecular gas constant and T is the absolute temperature. Equation 1 has been tested by experiment and shown to hold rigorously under ordinary conditions, but recent theoretical developments have shown that this is more or less accidental. Equation 1 is derived by the use of the statistics of Boltzmann. We now know that the correct statistics for neutral molecules are those of Bose and Einstein. Both methods of calculation lead to the same limiting form of the distribution law, equation 1, for a gas at high temperatures and low pressures, but the Bose-Einstein method gives the correct result under all conditions and is free from certain inconsistencies that mar the Boltzmann statistics.

Equation 1 is often written:

$$N_n = NKp_n e^{-\frac{\epsilon_n}{kT}} \tag{2}$$

where p_n is the weight factor of the nth energy level. If p_n has a value different from unity it means that the level is degenerate, that is, that under a perturbing field, the molecules will be separated into classes which differ from each other slightly in energy. In other words, p_n is the multiplicity of the nth level. It will often be simpler for our purposes to assign to a level with the multiplicity p_n the designations n, $n+1$, etc., with the understanding that the energies ϵ_n, ϵ_{n+1}, etc., become equal to each other in the absence of a perturbing field.

THE BOSE-EINSTEIN STATISTICS

Let p_i be the multiplicity of a level with energy ϵ_i. Then the number of different ways in which N_i molecules may be distributed among the p_i levels is

$$\frac{(N_i + p_i)!}{N_i!\, p_i!}$$

$N_i!$ appears in the denominator because in the Bose-Einstein statistics the interchange of like molecules is without significance, and p_i appears in the denominator, because the interchange of levels can have no physical meaning. The total number of possible arrangements of N molecules among a series of energy levels is given by the continued product of terms

$$' = \prod_{i=0}^{\infty} \left[\frac{(N_i + p_i)!}{N_i!\, p_i!} \right] \tag{3}$$

The most probable values for N_0, N_1, etc., are those which will make P a maximum. This condition may be stated for our purposes most conveniently,

$$\delta \ln P = 0 \tag{4}$$

Expanding equation 3 and remembering that p_i is a constant, we have[3]

$$\sum_{i=0}^{\infty} \ln \left(1 + \frac{p_i}{N_i} \right) \delta N_i = 0 \tag{5}$$

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[3] When N is a large number

$$N! = N^N e^{-N} \sqrt{2\pi N}$$

For the calculations in this paper a sufficient approximation is obtained by writing

$$N! = N^N e^{-N}$$

Taking logarithms, equation 3 becomes

$$\ln P = \Sigma \ln \frac{(N_i + p_i)^{N_i + p_i} e^{-(N_i + p_i)}}{N_i^{N_i} p_i^{p_i} e^{-N_i} e^{-p_i}}$$

This becomes

$$\delta \ln P = \Sigma (N_i + p_i) \ln (N_i + p_i) - \Sigma N_i \ln N_i - \Sigma p_i \ln p_i$$

Since the total number of molecules and the total energy is constant, we have two further conditions

$$\Sigma N_i = N \tag{6}$$
$$\Sigma \epsilon_i N_i = E \tag{7}$$

which may be written

$$\Sigma \delta N_i = 0 \tag{8}$$
$$\Sigma \epsilon_i \delta N_i = 0 \tag{9}$$

If, following the custom of the mathematician, we multiply equations 8 and 9 by undetermined multipliers, α and β, and add them to equation 5 we have

$$\sum \left[\ln \left(1 + \frac{p_i}{N_i} \right) + \alpha + \beta \epsilon_i \right] \delta N_i = \tag{10}$$

Since for the maximum value of P the δN's are arbitrary we may choose values for the δN's, α, and β so that all terms vanish except the one which contains N_n.

$$\ln \left(1 + \frac{p_n}{N_n} \right) + \alpha + \beta \epsilon_n = 0 \tag{11}$$

This may be rewritten

$$1 + \frac{p_n}{N_n} = e^{\alpha + \beta \epsilon_n} \tag{12}$$

or

$$N_n = e^{\alpha + \beta \epsilon_n} - 1 \tag{13}$$

It turns out that for a gas at ordinary temperature and pressure e^{α} is very large compared to unity so that we may write

$$N_n = p_n e^{-\alpha} e^{-\beta \epsilon_n} \tag{14}$$

Remembering that N_i is varied and p_i is constant we get

$$\delta \ln P = 0 = \Sigma \delta N_i + \Sigma \ln (N_i + p_i) \delta N_i - \Sigma \delta N_i - \Sigma \ln N_i \delta N_i$$

This leads to equation 5 above.

A given set of values for N_0, N_1, N_2, etc., is called a distribution. The number of ways in which this distribution may be obtained, counting as one those which differ only by permutations of like molecules, will be referred to as the number of arrangements.

β is to be identified with $\dfrac{1}{kT}$ so that we have the general form of equation 1

$$N_n = p_n e^{-\alpha} e^{-\frac{\epsilon_n}{kT}} \qquad (15)$$

THE ENERGY LEVELS OF A MONATOMIC GAS

The experiments of Stern on the reflection of molecules from crystal surfaces show that the motion of a free particle may be represented by a DeBroglie wave of wave length $\lambda = \dfrac{h}{mv}$. Just as there is a limitation on the number of stationary acoustical waves that can exist in a box so is there a precisely analogous limitation on the number of possible DeBroglie waves in an enclosure. The effect of this is to limit the momentum and hence the translational energy of the molecule to certain discrete values. The result is most easily obtained from the Schroedinger equation

$$\nabla^2 \psi + \frac{8\pi^2 m}{h^2}(E - W)\psi = 0 \qquad (16)$$

W, the potential energy, is zero throughout the box containing the gas and ψ must vanish at the walls. The solution of the above differential equation is

$$\psi = \sin \frac{n_1 \pi x}{l} \sin \frac{n_2 \pi y}{l} \sin \frac{n_3 \pi z}{l} \qquad (17)$$

where l is the length of one edge of the cubical box. On substitution of equation 17 in equation 16 it is seen that the permitted values of E are

$$E = \epsilon_n = \frac{h^2}{8ml}(n_1^2 + n_2^2 + n_3^2) \qquad \cdot \qquad (18)$$

Here h is Planck's constant, m is the mass of the molecule and n_1, n_2 and n_3 are three quantum numbers corresponding to the three degrees of freedom of the molecule. These numbers are restricted to integral values. The factor p_n in equation 15 represents the number of possible ways in which a given value

ϵ_n may be obtained by using different integral values for n_1, n_2 and n_3. For the simple form, equation 15, it is possible to evaluate $e^{-\alpha}$.

In order to do this it is necessary to note the significance of p_n. p_n is the number of different ways that a given energy

$$\epsilon_n = \frac{h^2}{8ml^2}(n_1^2 + n_2^2 + n_3^2) \tag{19}$$

may be represented as a sum of the squares n_1^2, n_2^2, n_3^2. Physically these different representations correspond to the different directions of velocity possible for a molecule of kinetic energy, ϵ_n, inside the box.

It is not possible to express p_n as a simple function of ϵ_n, and it is not necessary to do this to obtain e^{α}. If we sum equation 15 over all values of n we only need to remember that the exponential term is to be summed over all integral values of n_1, n_2, n_3, and p_i drops out. We have

$$\sum N_i = N = e^{-\alpha} \sum_{n_1} \sum_{n_2} \sum_{n_3} e^{-\frac{h^2(n_1^2 + n_2^2 + n_3^2)}{8ml^2kT}} \tag{20}$$

This may be written

$$Ne^{\alpha} = \sum_{n_1} e^{-\frac{h^2 n_1^2}{8ml^2kT}} \sum_{n_2} e^{-\frac{h^2 n_2^2}{8ml^2kT}} \sum_{n_3} e^{-\frac{h^2 n_3^2}{8ml^2kT}} \tag{21}$$

As

$$\frac{h^2}{8ml^2kT}$$

approaches zero the value of

$$\sum_n e^{-\frac{h^2 n^2}{8mlkT}}$$

approaches

$$\sqrt{\frac{2\pi ml^2kT}{h^2}}$$

and equation 21 becomes

$$Ne^{\alpha} = \left(\frac{2\pi ml^2kT}{h^2}\right)^{\frac{3}{2}} \tag{22}$$

But

$$l^3 = V \tag{23}$$

where V is the volume of the box.   Hence[4]

$$e^\alpha = \left(\frac{2\pi mkT}{h^2}\right)^{\frac{3}{2}} \frac{V}{N} \tag{24}$$

Equation 13 may be written in the form of equation 15 when e^α is large compared to unity, and by reference to equation 24 we see that this is the case for a gas at ordinary temperatures and pressures.

The general form of the distribution law for a monatomic gas may therefore be written

$$N_n = \frac{N p_n e^{-\frac{\epsilon_n}{kT}}}{\left(\frac{2\pi mkT}{h^2}\right)^{\frac{3}{2}} V} \tag{25}$$

which is the standard form in which we shall write all distribution laws.[5]

THE DISTRIBUTION LAW FOR DIATOMIC GASES

The general form of the distribution for diatomic gases must take account of the internal energy of the molecule.   Actually

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[4] The author is indebted to Mr. R. H. Ewart for this method of evaluating e^α.

[5] In the sketchy derivation given above, we have only indicated that equation 25 represents the most probable distribution of molecules.   It can be shown mathematically that the number of arrangements which gives the distribution of equation 25 is so much greater than the number of arrangements corresponding to any distribution differing appreciably from equation 25, that the total number of all other arrangements is negligible when compared with the number of arrangements corresponding to the distribution of equation 25.

The fundamental postulate of the Bose-Einstein statistics is that the permutation of like molecules does not produce a new arrangement of molecules.   In the Boltzmann statistics each permutation of molecules is counted as a new arrangement.   The fact that the two kinds of statistics give the same limiting form of the distribution law must be regarded as a coincidence.   The distribution law (7) is derived for a constant total energy.   It remains to show that the total energy is related in some simple way to the absolute temperature.   This may be done either by use of the laws of thermodynamics or by an arbitrary definition of absolute temperature in terms of the average translational energy of a gas molecule at higher temperatures.

of course at any instant a molecule possesses a certain discrete energy, but this energy may be represented as a sum of translational, rotational, vibrational and electronic terms. Thus,

$$\epsilon_n = \epsilon_{n(tr)} + \epsilon_{n(r)} + \epsilon_{n(v)} + \epsilon_{n(e)} \tag{26}$$

For example, in addition to possessing a certain discrete translational energy a molecule may at a given instant exist in particular rotational, vibrational and electronic states.

Corresponding to the multiplicity of each state or level there will be an integral weight factor. The general distribution law for a diatomic molecule thus becomes

$$N_n = NK p_{n(tr)} p_{n(r)} p_{n(v)} p_{n(e)} e^{-\frac{\epsilon_{n(tr)} + \epsilon_{n(r)} + \epsilon_{n(v)} + \epsilon_{n(e)}}{kT}} \tag{27}$$

In so far as the distributions of these energies are independent of one another, distribution equations may be written for particular forms of the energies. Thus,

$$N_n = NK p_{n(r)} e^{-\frac{\epsilon_{n(r)}}{kT}} \tag{28}$$

represents the distribution of diatomic molecules among the various rotational levels. The internal energy of the molecule is quite independent of the translational energy, but the distribution of the various rotational, vibrational and electronic energies may only be considered independent as a first approximation. The reasons for this will be noted later.

GENERAL PROPERTIES OF THE DISTRIBUTION EQUATION

The partition function

By summing equation 2 over all energy levels we obtain

$$\sum_{i=0}^{\infty} N_i = N = NK \sum_{i=0}^{\infty} p_i e^{-\frac{\epsilon_i}{kT}} \tag{29}$$

From this equation we see that

$$= \frac{1}{\sum_{i=0}^{\infty} p_i e^{-\frac{\epsilon_i}{kT}}} \tag{30}$$

and equation 2 becomes

$$N_n = \frac{N p_n e^{-\frac{\epsilon_n}{kT}}}{\sum_{i=0}^{\infty} p_i e^{-\frac{\epsilon_i}{kT}}} \tag{31}$$

The summation

$$\sum_{i=0}^{\infty} p_i e^{-\frac{\epsilon_i}{kT}}$$

has been called by Planck the "Zustandsumme." This is not a convenient word for an English-speaking person and it has no satisfactory translation, but R. H. Fowler has suggested that it

$e^{-\frac{\epsilon}{kT}}$

FIG. 2. GRAPHICAL REPRESENTATION OF THE DISTRIBUTION LAW

be called the "partition function" and we shall use this term for this very important expression to which we shall be constantly referring in this paper.

The graphical representation of the partition function

In figure 2 is a graphical representation of a partition function. The various terms of the summation $e^{-\frac{\epsilon_i}{kT}}$ are plotted as ordinates against the energy divided by kT, as abscissas. The partition function is obviously the sum of the lengths of the ordinates to infinity. This is a very convenient graphical representation.

Since the spacing of the ordinates is proportional to the spacing of the energy levels it is convenient to turn the diagram (figure 2) around and plot it as in figure 3. The plot is now similar to figure 1. The spacings of the energy levels are multiplied by the
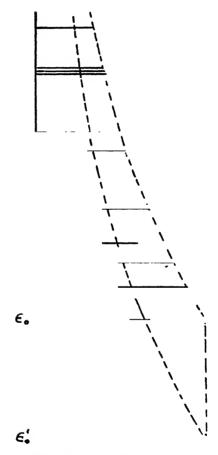
FIG. 3. GRAPHICAL REPRESENTATION OF THE PARTITION FUNCTION

constant factor $\frac{1}{kT}$, but this causes no difficulty since the scale is arbitrary. The distribution of the molecules among the various levels is easily visualized, since the number of molecules in any

level is proportional to the length of the level. Degenerate levels are represented simply as multiple levels, the lines being drawn very close together. The effect of temperature on the distribution is shown in figure 4, where the spacing of the levels
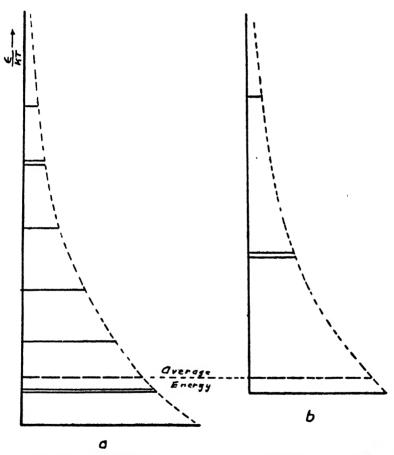
FIG. 4. GRAPHICAL REPRESENTATION OF PARTITION FUNCTIONS AT DIFFERENT TEMPERATURES

The average energy is taken as the zero in each case

is increased, due to a small value of kT corresponding to a lower temperature. The bounding curve represented by the dotted line remains unchanged.

The zero of energy is always arbitrary. The effect of a change in this arbitrary zero of energy is shown in figure 3. Unit length is always assigned to the level of zero energy. If we wish to take our zero of energy, not as the diagram is drawn but at a point ϵ_0^1 which lies a distance $\frac{\epsilon}{kT}$ below ϵ_0 we simply draw a new bounding curve starting with ϵ_0^1. Mathematically this is equivalent to adding the term $\frac{\epsilon}{kT}$ to each of the values $\frac{\epsilon_1}{kT}$, $\frac{\epsilon_2}{kT}$, etc. We now write the partition function,

$$\sum_{i=0}^{\infty} e^{-\frac{\epsilon_i + \epsilon}{kT}} = e^{-\frac{\epsilon}{kT}} \sum e^{-\frac{\epsilon_i}{kT}} \tag{32}$$

It is seen that the change in the zero point of energy is equivalent to multiplying the partition function by the term $e^{-\frac{\epsilon}{kT}}$. The value of the partition function depends upon the arbitrary zero of energy chosen. It must be remembered that the arbitrary zero of energy is not an energy level of the molecule, except as it may coincide with one of the levels.

The partition functions for various kinds of energy

By comparison of equation 25 with equation 31 we see that the partition function for the translational energy of a gas is[6]

$$\left(\frac{2\pi mkT}{h^2}\right)^{\frac{3}{2}} V$$

It has been stated above that the distributions of the rotational and vibrational energies of a diatomic molecule are practically independent of each other. The spacing of the rotational levels

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[6] The complete partition function for the atom of a monatomic gas must include the electronic levels to which the atom is excited in the emission of the line spectrum. The first excited state usually lies so far above the normal state that it need not be considered. However, except for ^1S states the normal levels of the atom are multiple, and a corresponding multiplicity is introduced into the partition function.

depends upon the moment of inertia, and the moment of inertia is not affected by the lower state of vibration. Neither does the rotation affect the vibrational frequency appreciably. On the other hand, electronic excitation usually changes both the moment of inertia and the vibrational frequency.

In the absence of electronic excitation, theoretical expressions can be obtained for the rotational and vibrational partition functions. The rotational partition function is

$$\sum_{n=0}^{\infty} (2n+1) e^{-\frac{n(n+1)h^2}{8\pi^2 IkT}}$$

The sum of this series approaches the limit

$$\frac{8\pi^2 IkT}{h^2}$$

when the term

$$\frac{h^2}{8\pi^2 IkT}$$

is small. For large values of n the moment of inertia I increases due to the stretching effect of the centrifugal force, but this effect is usually negligible. If the term

is not small the series must be summed term by term. For the vibrational partition function we have

$$\sum_{n=0}^{\infty} e^{-\frac{nh\omega}{kT}} = \frac{1}{1 - e^{-\frac{h\omega}{kT}}} \tag{33}$$

For the higher vibrational states the frequency ω does not remain constant so that an error is introduced. For the more complicated states of molecules, such as the $^3\Sigma$ which characterizes oxygen, or the $^2\Pi$ which characterizes the hydroxyl molecule, various types of multiplicity appear which must be taken into account. In any event the exact value of the partition function may always be calculated by a term by term summation over the energy levels which are given by an analysis of the band spectrum.

THE ENERGY AND HEAT CAPACITY

The total energy of any kind in a system of molecules in any form may be obtained readily if the distribution of that particular kind of energy is independent of other forms of energy. For example, the total rotational energy is given by the expression,

$$E_r = \sum_{i=0}^{\infty} N_i \epsilon_i = \frac{N \Sigma p_i \epsilon_i e^{-\frac{\epsilon_i}{kT}}}{\Sigma p_i e^{-\frac{\epsilon_i}{kT}}} \tag{34}$$

The value obtained for the energy depends naturally upon the particular arbitrary zero that is chosen for the energy. On the other hand the heat capacity is independent of the arbitrary zero of energy. The heat capacity is given by the expression,

$$\frac{\partial E}{\partial T} = \frac{N}{kT^2} \left[\frac{\Sigma p_i \epsilon_i^2 e^{-\frac{\epsilon_i}{kT}}}{\Sigma p_i e^{-\frac{\epsilon_i}{kT}}} - \left(\frac{\Sigma p_i \epsilon_i e^{-\frac{\epsilon_i}{kT}}}{\Sigma p_i e^{-\frac{\epsilon_i}{kT}}} \right)^2 \right] \tag{35}$$

The foregoing formulas are of course exact only under conditions of temperature and pressure where equation 15 is valid, that is, where the Bose-Einstein statistics give the same result as the classical Boltzmann statistics.

THE ENTROPY

The customary *a priori* definition of entropy is by the relation

$$S = K \ln P \tag{36}$$

Where P is the total number of possible arrangements of a system of molecules. For a system of molecules obeying the Bose-Einstein statistics we evaluate P as in equation 3 since, as has already been pointed out, the most probable distribution includes all but a negligible fraction of the total possible arrangements. From equation 3 therefore,

$$S = K \ln \prod_{i=0}^{\infty} \left(\frac{(N_i + p_i)!}{N_i! \, p_i!} \right) \tag{37}$$

Expanding the factorials as before we have

$$S = K \sum_{i=0}^{\infty} \ln\left(1 + \frac{p_i}{N_i}\right)^{N_i} + K \sum_{i=0}^{\infty} \ln\left(1 + \frac{N_i}{p_i}\right)^{p_i} \tag{38}$$

From equation 11 the first term of this expansion becomes, for one mole,

$$k \sum_{i=0}^{\infty} N_i\left(\alpha + \frac{\epsilon_i}{kT}\right) = R \ln\left[\left(\frac{2\pi mkT}{h^2}\right)^{\frac{3}{2}} \frac{V}{N}\right] + \frac{E}{T}$$

$$= R \ln\left[\left(\frac{2\pi mkT}{h^2}\right)^{\frac{3}{2}} \frac{V}{N} e^{\frac{3}{2}}\right] \tag{39}$$

where E is the total energy per mole $\left(= \frac{3}{2}RT\right)$. In order to evaluate the second term in this equation we may rewrite it,

$$k \sum_{i=0}^{\infty} N_i \ln\left(1 + \frac{N_i}{p_i}\right)^{\frac{p_i}{N_i}}$$

If $\frac{p_i}{n_i}$ is small, the value of the expression approaches unity and the logarithm is zero; if $\frac{p_i}{n_i}$ is large, the expression approaches the number e as a limit and the expression becomes

$$K \Sigma N_i = R \tag{40}$$

The value of the second term therefore increases from a limiting value of zero at 0°K. to R, at temperatures where equation 25 holds for a gas. The entropy of a monatomic gas is given exactly under ordinary conditions by the equation,

$$S = R \ln\left[\left(\frac{2\pi mkT}{h^2}\right)^{\frac{3}{2}} \frac{V}{N} e^{\frac{5}{2}}\right] \tag{41}$$

ROTATIONAL AND VIBRATIONAL ENTROPIES

We have seen that where the distribution of one form of energy is independent of other forms, a separate distribution equation can be written for this form of energy as equation 34. By the

same process of reasoning we can separate the terms involving the rotational or vibrational entropy. By substituting from equation 27 in equation 41 we obtain as the general expression for the entropy of a diatomic gas

$$S = R \ln\left[\left(\frac{2\pi mkT}{h^2}\right)^{\frac{3}{2}}\frac{V}{N}e^{1}\right] + \frac{E_r}{T} + R \ln \sum_{i=0}^{\infty} p_i e^{-\frac{\epsilon_i(r)}{kT}} + \frac{E_v}{T} + R \ln \sum_{i=0}^{\infty} e^{-\frac{\epsilon_i(v)}{kT}}$$

This equation may be written as a sum of entropy terms of which, for example, the rotational entropy is

$$S = R \ln \sum_{i=0}^{\infty} p_i e^{-\frac{\epsilon_i(r)}{kT}} + \frac{E_r}{T} \tag{43}$$

If we write

$$E = N_0 \bar{\epsilon} \tag{44}$$

where $\bar{\epsilon}$ is the average rotational energy per molecule, equation 42 may be written

$$S = R \ln \sum_{i=0}^{\infty} p_i e^{-\frac{\epsilon_i}{kT}} + R \ln e^{-\frac{\bar{\epsilon}}{kT}} = R \ln \sum_{i=0}^{\infty} p_i e^{-\frac{\epsilon_i - \bar{\epsilon}}{kT}} \tag{45}$$

The same transformation may be made for any form of energy which is independently distributed.

The entropy is seen to be equal to R times the logarithm of the partition function when the average energy per molecule is arbitrarily taken as the zero of energy. Reference to figure 4 will make this clear. In accordance with our graphical scheme of representation the zero of energy is a line of unit length. As the temperature approaches zero, the average energy per molecule approaches and coincides with the lowest level and the partition function takes on the value unity and the entropy becomes zero. It would be a case of *petitio principii* to assume that this was a demonstration of the absolute character of entropy, because we must remember that we assumed in our derivation of the equation for entropy that the additive constant was zero. However, this seems to be a very illuminating illustration of the significance of entropy. The entropy of a system of molecules is a measure

of the extent to which the molecules are distributed over different levels.

It is important to notice the effect of the weight factor p_n in cases where it is different from unity. This factor gives the multiplicity of the level and is taken care of in our graphical representation by actually representing the levels as multiplets. In the absence of a perturbing field these multiplets would coincide, but we may always assume that some perturbing field is present due to adjacent molecules. Under these conditions we may be sure that the multiple levels will be separate, and this has an important bearing on the entropy at $0°K$. of substances such as molecular oxygen or ortho-hydrogen. Each of these molecules has a resultant spin of one unit. The spin in the oxygen molecule is due to the electrons and in the ortho-hydrogen molecule it is due to the protons. In addition, the ortho-hydrogen molecule has an angular momentum of one unit in its lowest state. On account of the different orientations of the spin and angular momentum in the external field, which may always be assumed to be present with corresponding slight differences in energy, the lowest level for the oxygen molecule is threefold, and for the ortho-hydrogen molecule ninefold. As the temperature is lowered to near absolute zero, however, the molecules will be found to lie on the lowest level of the multiplet, which of coures gives a zero entropy.[7]

In table 1 are given the values of the entropy and heat capacity at constant volume as calculated from the data of the band spectra for a number of molecules. The calculations for a number of these molecules have been published elsewhere by various authors. The data calculated in this way, while in general agreement with the data obtained by direct thermal measurements, are more accurate. The entropy of the hydrogen compounds includes a term $R \ln 2$ for each atom of hydrogen in the molecule. This is to take account of the multiplicity produced by the proton spin which has been discussed above. No term is added for the nuclear spin of other elements such as chlorine,

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[7] Contrast Stern, T. E.: Proc. Roy. Soc. London **130A**, 367 (1931).

because the spin is not known with certainty.   In the case of hydrogen, the spin effect may be obtained by direct thermal measurements on the equilibrium mixture of ortho- and para-hydrogen.[8]   In figure 5 is given the heat capacity curve, as calculated by Bonhoeffer and Harteck (9) for hydrogen gas, in the presence of a catalyst for the interconversion of ortho- and para-molecules.   This curve rises to a very high peak at low temperatures, which gives a large contribution to the entropy and goes to make up the additional value of $R \ln 4$ which is to be included in the entropy of the hydrogen molecule.   Giauque and

TABLE 1

Entropies and heat capacities calculated from spectroscopic data[9]

SUBSTANCE	REFERENCES TO LITERATURE	ENTROPY 298°K. 1 ATMOSPHERE PER MOLE	HEAT CAPACITY AT CONSTANT VOLUME AT 298°K. PER MOLE
H_2	(1)	34.0	4.91
O_2	(2)	49.0	5.03
Cl_2	—	54.0	6.09
I_2	(3)	62.3	6.81
HCl	(4)	46.3	4.97
HBr	(5)	49.2	4.97
HI	(6)	51.1	4.97
CO	—	47.3	4.97
NO	(7)	50.4	5.13
OH	(8)	45.3	

Johnston (10) and R. H. Fowler (11) have calculated the entropy of the metastable 3 to 1 mixture of ortho- and para-hydrogen at low temperatures.   It is possible to do this, and the entropy will

[8] The nuclear spin of an atom makes the same contribution to the entropy per gram-atom in the elementary state and in compounds.   So far as practical calculations are concerned, therefore, this entropy may be included or omitted, provided only that the practice be consistent.

[9] The particular values given herewith were calculated by the members of the author's seminar in physical chemistry in 1929–30.

In stating values of the heat capacity for gases at temperatures and pressures where appreciable dissociation occurs, it is necessary to state whether the heat capacity is calculated for the *pure* diatomic gas or for an equilibrium mixture. The values given in this table are for the pure diatomic gas.

have significance for any equilibrium in which the relative proportions of the two kinds of molecules are not disturbed. The equilibrium between vapor and solid or liquid hydrogen is presumably such an equilibrium, although here it is possible that the vapor is not in equilibrium with a condensed phase of the same composition.
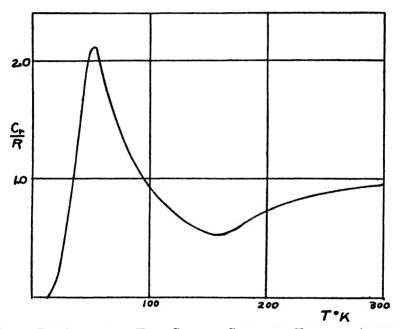
Fig. 5. The Rotational Heat Capacity Curve for Hydrogen Assuming Intercombination of Odd and Even States

The entropy of the electron

According to the new quantum mechanics the electron should have a weight factor of two in the vapor state because of its spin. This leads to an equation for the emission of electrons from hot filaments of the form

$$I = AT^2 e^{-\frac{b_0}{T}} \tag{68}$$

in which theoretical value of the constant A should be 120.4. Dushman (12) has shown that the best experimental value for this

constant A is 60.2, or one-half the required value. This might be taken to indicate that the electron does not have a statistical weight of two in the vapor state. Another possibility is that the reflection coefficient of electrons from filaments is about 0.5. It seems more probable, however, that some error exists in the emission data themselves. The problem requires further investigation and is an interesting one because it is the only way that has so far been proposed for a direct experimental proof of the existence of spin in the free electron.

The isotope effect in the entropy of chlorine

An interesting effect is to be predicted in the entropy of chlorine. Since there are present atoms of Cl_{35} and Cl_{37} we may expect an equilibrium of the following sort.

$$Cl_{35}Cl_{35} + Cl_{37}Cl_{37} = 2Cl_{35}Cl_{37} \tag{46}$$

If we assume the heat of dissociation of the three kinds of molecules to be exactly the same, then it follows from simple kinetic considerations that the chances of forming a heteronuclear molecule are twice as great as for a homonuclear molecule. As a result we should have at equilibrium

$$\frac{P^2_{Cl_{35}Cl_{37}}}{P_{Cl_{35}Cl_{35}} \, P_{Cl_{37}Cl_{37}}} = 4 \tag{47}$$

Since

$$\Delta F^\circ = -RT \ln K$$

and

$$\Delta H = 0$$

it follows that

$$\Delta S = R \ln 4 \tag{48}$$

or that the entropy of the heteronuclear molecule is greater by $R \ln 2$ than that of the homonuclear molecule. This result is confirmed by the quantum mechanics, which predicts that alternate rotational levels will be missing for homonuclear molecules. This effect has nothing to do with the entropy of mixing or with

nuclear spin effects. At low temperatures the equilibrium will be shifted in an irregular manner until finally at very low temperatures, where all rotational energy has been lost, it may be predicted that the entropy of homonuclear and heteronuclear molecules would be the same and the equilibrium constant of equation 47 will be unity. In this discussion the slight variation of the entropy with the masses of the different isotopes has been neglected.

CHEMICAL EQUILIBRIUM

In a general discussion of chemical equilibrium the liquid and solid phases must be considered. While it is beyond the scope of this article to discuss them, partition functions exist for the liquid and solid states as well as for the gas, and these partition functions are related in the same way to the thermodynamic functions as the partition functions for gases are.

In the following discussion we shall limit ourselves to equilibria in which the gaseous phase is present.

Let us consider first an equilibrium wholly within the gaseous phases. Let the equilibrium be of the form.

$$A = nA' \tag{49}$$

For each species of molecule present there exists a partition function. Let us suppose a volume V to contain N molecules of A and N_1 molecules of A'. The condition for equilibrium is that the proportions of N and N_1 in the vessel must be such that the maximum number of possible arrangements of the two species is possible. If P_A is the total possible number of arrangements of A, and $P_{A'}$ the total number of arrangements of A', then the total number of arrangements of both species is the product $P_A P_{A'}$. The condition for equilibrium is that this product shall be a maximum, and this condition may be conveniently expressed by taking logarithms and differentiating

$$\delta \ln P_A + \delta \ln P_{A'} = 0 \tag{50}$$

It turns out that P_A likewise $P_{A'}$ are represented by equations,

$$P_A = Q_A^N \tag{51}$$

$$P_{A'} = Q_{A'}^{N_1} \tag{52}$$

where Q_A and $Q_{A'}$ do not depend upon the total number of molecules present but only upon the number of molecules of each species per unit volume respectively.

We may now write

$$\ln P_A P_{A'} = N_1 \ln Q_A + N_2 \ln Q_{A'}. \tag{53}$$

If we now imagine the number of molecules to be varied by transforming a small number δN_1 molecules of A into δN_1 molecules of A′, the concentration of N and N_1 will not be affected appreciably and Q_{A_1} $Q_{A'}$ will remain constant. Equation 50 becomes therefore,

$$\delta N \ln Q_A + \delta N_1 \ln Q_{A'} = 0 \tag{51}$$

Remembering that

$$\delta N = n \delta N_1 \tag{52}$$

we have

$$\ln Q_A = n \ln Q_{A'} \tag{53}$$

or

$$Q_A = Q_{A'}^n. \tag{54}$$

Now the number of possible arrangements per molecule is measured by the partition function. We can see intuitively that this must be so since the number of possible arrangements of a molecule must depend upon the number of energy levels and also, of course, on the values of the energy for each level in precisely the way that the partition function does.

The statement can be demonstrated rigorously however, by considering the expansion of equation 37 for the total number of arrangements of a gas. This expansion leads to two varieties of terms. One of these terms is made up of the product of the partition functions for the various forms of energy divided by the total number of molecules N. The second term arising from the expansion is the total energy of N molecules, divided by the absolute temperature. The term R for example in equation 40 is equal to $\dfrac{PV}{T}$, and represents the potential energy of the gas, due

to the volume occupied under an external pressure, divided by the absolute temperature. This energy term appears in the expression for the total number of arrangements as an exponential multiplier $\left(e^{\frac{\bar{\epsilon}}{kT}}\right)$. The whole expression is the partition function as expressed in equation 45. If we multiply by $\left(e^{-\frac{\bar{\epsilon}}{kT}}\right)$ we refer the partition function to its lowest energy level as zero. This is permissible, since the zero of energy is arbitrary. Only in comparing two partition functions we must refer both functions to the same zero of energy and this may be done by multiplying one partition function by $e^{-\frac{\epsilon_0}{kT}}$, as is demonstrated in equation 32 and figure 3.

The condition for the equilibrium (equation 49) may be written down by substituting in equation 54 the partition functions for A and A' divided by N and N_1 respectively.

$$\left(\frac{2\pi m_A kT}{h^2}\right)^{\frac{3}{2}}\frac{V}{N}\Sigma p_i e^{-\frac{\epsilon_i}{kT}} = \left[\left(\frac{2\pi m_{A'} kT}{h^2}\right)^{\frac{3}{2}}\frac{V}{N}\Sigma p_i e^{-\frac{\epsilon_i'}{kT}}\left(e^{-\frac{\epsilon_0}{kT}}\right)\right]^n \quad (55)$$

The requirement that both partition functions be referred to the same zero is met by multiplying the second term of the equation by $e^{\frac{\epsilon_0}{kT}}$ where ϵ_0 is the difference between the lowest energy level of A and the lowest energy level of A' (figure 6). By taking logarithms and multiplying by R we obtain

$$R\ln\left[\left(\frac{2\pi m_A kT}{h^2}\right)^{\frac{3}{2}}\frac{V}{N}\Sigma p_i e^{-\frac{\epsilon_i}{kT}}\right] = nR\ln\left[\left(\frac{2\pi m_{A'} kT}{h^2}\right)^{\frac{3}{2}}\frac{V}{N}\Sigma p_i e^{-\frac{\epsilon_i}{kT}}\right] - \frac{nN_0\epsilon_0}{T} \quad (56)$$

The first two terms of this equation are respectively $S_1 - \dfrac{H_1}{T}$ and $n\left(S_2 - \dfrac{H_2}{T}\right)$, where S_1 and S_2 are the molal entropies and H_1 and H_2 the molal heat contents, referred to 0°K., for A and A', respectively. The last term is $\dfrac{\Delta H_0}{T}$. Since

$$\Delta H = \Delta H_0 - H_1 + nH_2$$
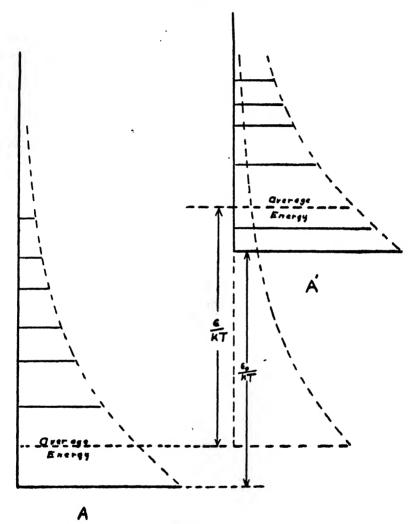
FIG. 6. PARTITION FUNCTIONS FOR TWO STATES A AND A' IN EQUILIBRIUM

we have

$$\Delta S = \frac{\Delta H}{T} \tag{57}$$

which is the fundamental thermodynamics condition for equilibrium.

One of the simplest types of gaseous equilibria is the equilibrium between the $^2\Pi_{\frac{1}{2}}$ and the $^2\Pi_{\frac{3}{2}}$ states of a molecule such as hydroxyl. For this equilibrium $n = 1$ and we have from equation 55

$$\frac{N_1}{N} = \frac{\left(\Sigma p_i e^{-\frac{\epsilon_i}{kT}}\right)_{\pi_{\frac{1}{2}}} e^{-\frac{\epsilon_0}{kT}}}{\left(\Sigma p_i e^{-\frac{\epsilon_i}{kT}}\right)_{\pi_{\frac{3}{2}}}} \tag{58}$$

A specific example of the equilibrium (equation 49) is a dissociation, for example

$$I_2 = 2\,I \tag{59}$$

Here the condition (equation 55) becomes

$$\left(\frac{2\pi m_2 kT}{h^2}\right)^{\frac{3}{2}} \frac{V}{N} \Sigma_{I_2} p_i e^{-\frac{\epsilon_i}{kT}} = \left[\left(\frac{2\pi m kT}{h^2}\right)^{\frac{3}{2}} \frac{V}{N_1} \Sigma_I p_i e^{-\frac{\epsilon_i}{kT}}\left(e^{-\frac{\epsilon_0}{kT}}\right)\right]^2 \tag{60}$$

where $m_2 = 2m$. Making the substitution

$$\frac{V}{N} = \frac{kT}{P} \tag{61}$$

equation 60 becomes,

$$\ln \frac{P_1^2}{P} = \ln K - \frac{\Delta H_0}{RT} + \frac{5}{2}\ln(kT) + \ln\left(\frac{\pi m}{h^2}\right)^{\frac{3}{2}} - \ln \Sigma(2n+1)\,e^{-\frac{n(n+1)h^2}{8\pi^2 I kT}} -$$

$$\ln \Sigma e^{-\frac{n h \omega}{kT}} + \ln \Sigma_I p_i e^{-\frac{\epsilon_i}{kT}} \tag{62}$$

Here P is the partial pressure of the iodine molecules, P_1 the partial pressure of the iodine atoms, I the moment of inertia of the molecule, ω the vibrational frequency and

$$\Sigma_i p_i e^{-\frac{\epsilon_i}{kT}}$$

the partition function for the electronic levels of iodine atom. ΔH_0 is equal to the spectroscopic heat of dissociation. Since half the rotational levels are missing for the iodine molecule we have for the rotational partition function,

$$\frac{1}{2}\left(\frac{8\pi^2 I kT}{h^2}\right)$$

The lowest level of the iodine atom has a multiplicity of 4 and the next levels are so high that they may be neglected. Hence we have (13)

$$\ln K = -\frac{\Delta H_0}{RT} + \frac{3}{2}\ln\left(\frac{\pi mkT}{h^2}\right) - \ln\left(\frac{8\pi^2 I}{h^2}\right) + \ln\left(1 - e^{-\frac{h\omega}{kT}}\right) + 5\ln 2 \quad (63)$$

Another simple illustration of equilibrium is the vapor pressure of a crystal whose vapor is monatomic. For this equilibrium

$$\Sigma_{crystal} = \left(\frac{2\pi mkT}{h^2}\right)^{\frac{3}{2}} \frac{V}{N} e^{-\frac{\epsilon_0}{kT}} \quad (64)$$

This gives the equation

$$\ln P = -\frac{\Delta H}{RT} + \frac{5}{2}\ln T - \quad_R \quad \cdot \ln\left[\left(\frac{2\pi m}{h^2}\right)^{\frac{3}{2}} k^{\frac{5}{2}}\right] + \frac{5}{2} \quad (65)$$

The term

$$\ln\left[\left(\frac{2\pi m}{h^2}\right)^{\frac{3}{2}} k^{\frac{5}{2}}\right]$$

is the "true" chemical constant of Nernst.

The hydroxyl equilibrium

The reaction

$$H_2O_x + \tfrac{1}{2}O_2 = 2\,OH \quad (66)$$

is an example of one for which it is possible to calculate the thermal equilibrium from data obtained chiefly from spectroscopy. For this reaction ΔH is estimated to be 18,000 calories. The entropy for water vapor at 298°K. is estimated to be 46.9 E.U. Hence $\Delta S = 19.6$. We have therefore

$$\Delta F° = 18,000 - 19.6\,T \quad (67)$$

At ordinary temperatures there will be very little hydroxyl at equilibrium. At higher temperatures there will probably be only small changes in ΔH and ΔS, so that the equilibrium will be shifted toward the hydroxyl. It is possible that $\Delta F°$ may be negative at 1000°K.

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BONDING POWER OF ELECTRONS AND THEORY OF VALENCE[1]

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I. INTRODUCTION

Valence is commonly classified under the headings of polar and non-polar valence, and of primary and secondary valence. It has, however, long been evident that no sharp lines can be drawn in general between these various kinds of valence. In fact, the concept of valence itself is one which should not be held too sacred.

This paper will have to do principally with non-polar valence. Before trying to investigate the nature of this, it may be well to analyze the meaning of such a statement as "the valence of carbon is four." This meaning can be well illustrated by considering the series of conceivable compounds CH, CH_2, CH_3, CH_4, CH_5, CH_6, and so on. Of these, only CH_4 is stable in the ordinary chemical sense. In this compound, the carbon atom exhibits a valence of four, if unit valence is attributed to each hydrogen atom. In nearly all of its stable chemical compounds, in fact, carbon is attached to four univalent atoms or the equivalent.

[1] Presented at the eighty-first meeting of the American Chemical Society held at Indianapolis, Indiana, March 31, 1931, and since then somewhat revised and considerably enlarged. This paper is partly a review, partly a presentation of more or less new material. In a paper just published (in "Molekülstruktur," Leipziger Vorträge, 1931, p. 167. S. Hirzel, Leipzig), Herzberg has arrived independently at conclusions many of which are practically identical with those given here. The author is indebted to him for the opportunity of seeing the manuscript of his paper. A few points taken therefrom and not included in the original draft of the present paper but incorporated in the process of revision are so indicated in the text. In a paper also presented at the Indianapolis meeting, Urey (J. Chem. Education 8, 1114–32 (1931)) has discussed some of the points treated here, and partly in more detail.

What is wrong with the chemically unstable[2] molecules CH, CH_2, CH_3, CH_5, CH_6? There is really an essential difference between the cases of CH, CH_2, CH_3, and those of CH_5, CH_6, and so forth. An individual molecule of the first group is stable in the sense that a fairly large energy would be required to pull off a hydrogen atom ("physical stability[2]"), while CH_5 and CH_6 are presumably unstable even in this sense, just as a molecule composed of two helium atoms is unstable. The chemical instability of such a molecule as CH is, however, of an entirely different character from that of CH_6. The trouble with CH is not that the carbon and hydrogen atoms will not remain together, but that they can not keep other atoms away. Nevertheless the CH molecule is well known from the spectra of flames and of electric discharges, where artificial means are used to tear off the other atoms with which the CH normally unites.

This example illustrates two principal functions of valence theory, namely, to account for the existence of chemical binding, and to account for the phenomenon of saturation of valences.

II. THEORIES OF VALENCE

The Lewis theory

In the valence theory developed by Lewis and extended by Langmuir and others, atoms are supposed to be held together practically always by pairs of electrons, one electron usually coming from each atom. According to this theory, the electrons in each molecule seem tacitly to be considered as divided into two classes—the bonding electrons, nearly always in pairs, which actively hold the molecule together, and the remaining electrons, which may be called non-bonding electrons, since they are supposed to play an inert, or at least a minor, rôle in binding the atoms.

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[2] The term "molecular stability" as contrasted with "chemical stability" was used by Mulliken: Phys. Rev. **32**, 189 (1928). Herzberg in his new paper (cf. footnote 1) uses the term "physical stability" instead.

The theory of bonding and anti-bonding electrons and of bonding power

Another conception, which seems to correspond in a more natural way to our knowledge of the structure of diatomic molecules based on the interpretation of their spectra, is that we must assume not only bonding and non-bonding electrons, but also anti-bonding electrons, i.e., electrons which actively oppose a union of the atoms (1). More generally still, one may assign to each type of electron in a molecule a "bonding power," which may have any value, positive, negative, or zero, within a certain range (1). These conceptions first grew out of a development of the fundamental work of Hund (2) on the formation of diatomic molecules from atoms. Later on, examples will be given of the action of bonding and anti-bonding electrons in such molecules as NO, O_2, and F_2.

But the empirical rules of non-polar valence, which of course summarize the usual facts of combining ratios in stable non-polar compounds, suggest that the electrons in polyatomic molecules usually act in a more standardized way, classifying themselves quite definitely under the two headings "bonding" and "non-bonding." Herzberg (3) has, however, suggested that these rules of valence can be accounted for equally well in terms of bonding and anti-bonding[a] electrons. He points out that if each anti-bonding electron or electron-pair more or less exactly counterbalances a bonding electron or electron-pair, then the molecule behaves just as if the number of bonding pairs, i.e., the number of chemical bonds, were equal to the difference in the numbers of bonding and anti-bonding pairs.

If the numbers of bonding and anti-bonding electrons are equal, then zero valence bonds are present. This is the case when the attempt is made to bring together two atoms of a rare gas. The fact that two such atoms vigorously resist being brought together indicates that, although the bonding and anti-bonding electrons have been spoken of as more or less equal

[a] Hund (3) uses the expressive term "loosening" (lockernde) electrons for what are here called anti-bonding electrons.

although opposite in effect, the anti-bonding electrons are really decidedly more powerful than the bonding ones.

Quantum theory and molecule formation

The rules of valence are really concerned with the question of what particular molecular forms are chemically the most stable. Although quantum mechanics has not yet reached the point of accounting in detail for all the facts of valence, it does very definitely give the solution of the more general problem of why it is that atoms are capable of forming molecules at all. Quantum theory, following experiment, demands the existence of discrete stationary states of energy, for molecules as well as for atoms. It shows further that in each such stationary state the electrons may be thought of as moving in what used to and may still with reservations, be called orbits. And finally it shows, in outline at least, how when two or more atoms come together, the orbits of their electrons can be altered in a perfectly continuous manner to give the appropriate electron orbits of the molecule (Hund (2)). In the case of diatomic molecules, as well as that of polyatomic molecules in which all the nuclei are on a straight line, the electron orbits can often be classified under such names as $1s\sigma$, $2p\sigma$, $3d\pi$, or $\sigma 1s$, $\sigma^* 1s$, $\sigma 2s$, and so on, much the same as the electron orbits in atoms can be classified as $1s$, $2s$, $2p$, and so on.[4]

Formation of H_2^+ and H_2 molecules

Examples of bonding and anti-bonding electrons

The problem of valence is really one of energy relations. If there were rules for determining the energies of all the different kinds of possible electron orbits in molecules, and comparing

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[4] For a brief discussion of the meaning of σ and π orbits, see Mulliken, R. S.: Chem. Reviews **6**, 532 (1929). For a more detailed discussion, cf. reference 4. The present discussion of London and Heitler's valence theory should be taken as superseding that given in this earlier review. The $U(r)$ curves and dissociation products of the CN and N_2^+ molecules are also, according to the present belief, of the author, different from those given in figure 6 of this earlier review. For revised $U(r)$ curves see reference 4.

them with those in atoms, the rules of valence should follow more
or less automatically. Suppose for instance an ordinary hydro-
gen atom and a hydrogen ion be allowed to come together. (In
emphasizing H_2^+, the author is in part following Herzberg's new
paper.) It is known, from quantum theory and experiment, just
how firmly the hydrogen atom electron, which is of the $1s$ type,
is bound. It is also known from quantum theory that when the
atom and ion approach in the right way, the electron orbit tends
to reach out and surround both nuclei, thereby becoming more
firmly bound because it is now attracted by two nuclei instead
of one. This goes on until the resulting attractive forces are
balanced by the repulsion between the two nuclei. This is the
simplest case of an electron acting as a bonding electron. The
shape of the orbit is greatly changed when the atom and ion
come together, but it can still be classified as of the $1s$ type. It
is usually called $1s\sigma$, although in the simple case of $1s$ electrons,
the "σ" really adds nothing to the meaning. For the change
which occurs when the H_2^+ molecule is formed we may write:

$$H(1s) + H^+ \rightarrow H_2^+(1s\sigma, {}^2\Sigma_g^+)$$

The changes in the electron orbits when molecules are formed
are usually not so simple as in this case. Even here, there is a
second, more complicated way in which the atom and ion may
approach each other. In this, the electron undergoes a gradual
change, which will be referred to as a promotion, from its original
1-quantum orbit to a 2-quantum orbit; more specifically, to a
$2p\sigma$ orbit.[4] The change occurs gradually as the distance between
the nuclei is decreased (4), and would not be complete until the
nuclei were completely united ("united-atom"), which of course
is in practice impossible because of their mutual repulsion. The
change to a $2p\sigma$ orbit, if completed, would involve a very large
increase in energy as compared with a $1s\sigma$ orbit, since in any
atom the 2-quantum orbits are always much less firmly bound
than the 1-quantum orbit. The net result is that when the atom
and ion approach in such a way that the electron is promoted to
$2p\sigma$, the energy of promotion, which increases gradually as the
nuclei are brought together, together with the energy of repulsion

of the nuclei, causes the atom and ion continuously to repel each other, except at very large distances, so that no molecule is formed. The 1-quantum (1s) electron which would become $2p\sigma$ acts here as a typical anti-bonding electron. For the change which occurs when the atom and ion come together in this case we may write:

$$H(1s) + H^+ \rightarrow H_2^+(2p\sigma,\,{}^2\Sigma_u{}^+)$$

What happens to the original orbit as the atom and ion approach may be described in more detail approximately as follows. For $r = \infty$, the electron moves in 1-quantum orbit about one of the two nuclei. As r is decreased, the shape of the orbit is somewhat modified, in such a way that in the $1s\sigma$ case the electron spends more than half its time on the side of the original nucleus toward the other nucleus, or in the $2p\sigma$ case, on the side away from the other nucleus. Also, after going around the first nucleus a number of times, the electron jumps to the second nucleus; after usually about the same length of time, it jumps back to the first nucleus, and so on. As r is further diminished, the orbit becomes more and more deformed, and at the same time the jump frequency becomes larger and larger compared with the frequency of motion of the electron in its orbit around either nucleus. Finally when r is small enough—as is true in H_2^+ when r is equal to its equilibrium value—the two frequencies are of the same order of magnitude and the electron may best be thought of as moving in a single complicated orbit around both nuclei.

In the case of two neutral hydrogen atoms, just as in the case of $H + H^+$, there are two ways in which the atoms can approach each other. In the one mode of interaction, both electrons become $1s\sigma$ electrons and so act as bonding electrons, forming an ordinary stable H_2 molecule. In the other, one electron becomes $1s\sigma$ but the other is promoted to $2p\sigma$. The energy of promotion is so large in this case that it more than balances the energy gained by the increased firmness of binding of the $1s\sigma$ electron, and no stable molecule is formed. The changes which occur in the two cases when two hydrogen atoms come together may be written:

$$H(1s) + H(1s) \rightarrow H_2(1s\sigma^2,\,{}^1\Sigma_g{}^+)$$
$$H(1s) + H(1s) \rightarrow H_2(1s\sigma\,2p\sigma,\,{}^3\Sigma_u{}^+)$$

The heat of dissociation of the stable H_2^+ molecule held together by one $1s\sigma$ electron has been calculated with a fair degree of accuracy by quantum theory, and is about 61,000 calories per mole. This is approximately half as large as the heat of dissociation (103,000 calories) of H_2, which is held together by two $1s\sigma$ electrons. This fact indicates that to a first approximation each $1s\sigma$ electron acts independently as a bonding electron in H_2 (cf. also the next section).

Calculation method of Heitler and London

It should be pointed out that the method used above, which really amounts to an interpolation between separated-atoms and united-atom, can not always be relied on to determine whether or not a stable molecular state can be formed from two atoms interacting in a given way, although in most cases it gives good results. In the above case of $H_2(1s\sigma\ 2p\sigma,\ ^3\Sigma)$, for example, there might *conceivably* be a considerable attraction between the atoms at large distances, leading to the formation of a fairly stable molecule, in spite of the fact that at least at smaller distances the large promotion energy of $2p\sigma$ gives assurance of a strong repulsation. In other words, the process of promotion might conceivably at large distances not proceed fast enough to produce a repulsion.

But Heitler and London (5) have introduced a very valuable method of calculation, which shows definitely in the case of H + H that for $(1s\sigma)^2$ there is attraction, for $(1s\sigma)\ (2p\sigma)^3\Sigma$ repulsion, at all distances,—except that there is a superposed small van der Waals' attraction at very large distances. A similar result is found for $1s\sigma$ and $2p\sigma$ in H_2^+ (6). The method of Heitler and London consists in the application of the perturbation theory of quantum mechanics to the problem of calculating the energies of interaction of atoms. This *method* of Heitler and London should be carefully distinguished from the *valence theory* of Heitler and London, which will be discussed below.

In this and other simple cases in diatomic molecules, the method of Heitler and London makes it possible to calculate approximate values of the heat of dissociation, equilibrium distance be-

tween the nuclei, and other constants of stable molecular states, and to predict which of the various states that can in general be formed from two normal atoms should be attractive and which repulsive. The necessary calculations promise, however, to be rather complicated in all but the simplest cases. The method also fails to give a detailed insight into the nature of the changes which take place in the electron orbits when atoms come together.

In the method which is emphasized in this review, quantitative information and predictions as to the energies of interaction of two atoms and as to molecular constants are based mainly on empirical spectroscopic data (atomic and molecular). With the help of quantum theory, there is obtained a rather intimate picture of the changes which occur in the electron orbits when atoms unite.

Application of Heitler and London method to H_2 and H_2^+

Lack of fundamentalness, for valence theory, of the establishment of symmetrical relations between electrons during molecule-formation

In order to dispel what, in the author's opinion, are some misconceptions that have arisen in connection with the work of Heitler and London on molecule-formation and valence theory, and for other reasons, it will be of value to consider in some detail certain features of the Heitler and London method as applied to H_2^+ and H_2. (*This section can best be omitted,* or at least postponed, *by the casual reader.*)

For the energy change ΔW which occurs when an H atom and an H^+ ion come together, their method (6) gives the following expressions:

$$\Delta W = e^2/r - \frac{e^2 I_1}{1 + S} - \frac{e^2 I_2}{1 + S} \quad (\Delta W < 0 : 1s\sigma)$$

$$\Delta W = e^2/r - \frac{e^2 I_1}{1 - S} + \frac{e^2 I_2}{1 - S} \quad (\Delta W > 0 : 2p\sigma)$$

Here e^2/r is the energy of repulsion of the nuclei, while $e^2 I_1$ is the energy of attraction which the hydrogen ion would have for the

electron of the hydrogen atom, as calculated by quantum mechanics, if the electron orbit, or better, wave-function, were completely undeformed by the approach of the ion. The sum $e^2/r - e^2I_1$, commonly called the "Coulomb energy," gives at all values of r a repulsion, which is, however, slight except at small r values. The quantity S in the equations is small at large r values but approaches unity as $r \to 0$ (6).

The quantity e^2I_2 is the "exchange" (or "resonance") energy. The name "exchange energy" corresponds to the fact that the existence of this term is connected with the exchange or jumping back and forth of the electron between the two nuclei, described above. This exchange energy, modified by the factor $1/(1 \pm S)$, when *physically interpreted* is evidently essentially the energy required for the deformation of the $1s$ atomic orbit in $H + H^+$ either into $1s\sigma$ of H_2^+ (negative exchange energy and ΔW, increased firmness of binding) or into $2p\sigma$ of H_2^+ (positive exchange energy and ΔW, decreased firmness of binding).

In the case of two neutral hydrogen atoms, the results are similar, but are somewhat complicated by the presence of two electrons. The method of Heitler and London (6) gives the following:

$$\Delta W = e^2/r - \frac{e^2(2I_1 - I_4)}{1 + S^2} - \frac{e^2(2SI_2 - I_6)}{1 + S^2} \quad (\Delta W < 0 : 1s\sigma^2)$$

$$\Delta W = e^2/r - \frac{e^2(2I_1 - I_4)}{1 - S^2} + \frac{e^2(2SI_2 - I_6)}{1 - S^2} \quad (\Delta W > 0 : 1s\sigma\, 2p\sigma)$$

As compared with H_2^+, the most important changes in the ΔW formulas, for values of r corresponding to equilibrium in the stable state $(1s\sigma)^2$, are (a) $2I_1$ and $2I_2$ appear in place of I_1 and I_2 because there are now two electrons, *each* of which is attracted by the other nucleus as well as by its own; (b) the new terms I_4 and I_6, which represent energy of mutual repulsion of the two electrons, appear.

The Coulomb energy, given by $e^2/r - e^2(2I_1 - I_4)$, and corresponding to the net energy change which would result from the mere overlapping, if this were possible, of two undeformed hydrogen atoms, would give rise here to a mild attraction at moderate r values and to repulsion at small r values.

The exchange energy $e^2(2SI_2 - I_6)$ now consists of two parts. (*1*) The quantity $2e^2SI_2$ corresponds to the exchange or jumping of the electrons between the two nuclei, just like the analogous term in H_2^+, except that now two electrons jump (simultaneously, as it happens). Modified by the factor $1/(1 \pm S^2)$, it gives essentially the direct energy changes resulting from the deformation of the atomic $1s$ orbits to become molecular $1s\sigma$ or $2p\sigma$ orbits. With a minus sign in the ΔW equation it gives increased binding energy $(1s\sigma^2)$; with a plus sign it gives decreased binding energy $(1s\sigma2p\sigma)$. (*2*) The quantity e^2I_6 corresponds to an exchange of orbits between the two electrons. Modified by the factor $1/(1 \pm S^2)$, it gives mainly the changes in the energy of mutual repulsion of the two electrons which are incidental to the orbit-deformations just mentioned under (*1*). In the case of both the states $(1s\sigma)^2$, $^1\Sigma$ and $(1s\sigma)(2p\sigma)$, $^3\Sigma$, the terms $\pm e^2I_6$ oppose and partially cancel the terms $\mp 2e^2SI_2$.[5]

The physical reasons why the terms e^2I_6 appear in both the ΔW equations with opposite sign to the terms $2e^2SI_2$ are probably as follows. (*1*) In the $(1s\sigma^2$ state, both orbits are relatively small, so that the mutual repulsion of the electrons is increased as compared with what one would get from the I_4 part of the Coulomb energy, which corresponds to the mutual repulsion energy of undeformed but overlapping atomic $1s$ orbits. In the $(1s\sigma)(2p\sigma)$, $^3\Sigma$ state, however, one orbit $(2p\sigma)$ becomes relatively large, the other $(1s\sigma)$ relatively small, so that the energy of repulsion of the electrons is decreased. (*2*) These effects, which are probably the main ones, are somewhat intensified by the fact that in every singlet state (resultant spin $S = 0$) of a two-electron system the electrons are symmetrically related or symmetrically "connected" (in the words of London, "symmetrisch verknüpft"), and in every triplet state ($S = 1$) they are antisymmetrically

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[5] A rough calculation shows that for $r = 1.5\ a_0$, corresponding to the equilibrium separation in H_2 as calculated by the Heitler and London method, the electronic repulsion term $\dfrac{e^2I_6}{1 + S^2}$ is 5 volts, while the primary orbit-deformation term $\dfrac{2e^2SI_2}{1 + S^2}$ is 14 volts, for the $(1s\sigma)^2$, $^1\Sigma^+$ state.

related. (It should perhaps be pointed out that the letter S is here used with an entirely different meaning than in the last few paragraphs. It has still a third meaning in symbols such as ^1S, ^2S, ^3S used elsewhere in this paper.)

Strictly speaking, one should not say here that the electrons are symmetrically or antisymmetrically related, but only that in respect to their position coördinates, such relations exist. Really, when their spins are included, electrons in atoms or molecules are always antisymmetrically related. But for the sake of simplicity, the word "electrons" will be used in this connection throughout this paper with the tacit understanding that it implies position coördinates only and neglects spins.

The effect of a symmetrical relation is to make the electrons keep on the average closer together than they otherwise would, while an antisymmetrical relation makes them keep farther apart. Hence, unless other indirect effects are important, a symmetrical relation increases the energy of repulsion of the electrons and so the total energy, while an antisymmetrical relation decreases it. It was so that Heisenberg first explained the fact that, for example, the energy of the $1s\ 2s$, ^1S state of the helium atom (electrons symmetrically related, spin $= 0$) is higher than that of the $1s\ 2s$, ^3S state (electrons antisymmetrically related, spin $= 1$).

Most writers on the subject, beginning with London and Heitler, have emphasized the fact that when a stable H_2 molecule ($1s\sigma^2$) is formed, a symmetrical relation is established between the two electrons of H + H. But from the preceding analysis of the meaning of the "exchange energy" in the formation of H_2^+ and H_2, it seems clear that the establishment of a symmetrical relation between two electrons in H_2 when a valence bond is formed, is purely incidental.[6] Indeed it seems evident that the

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[6] Two electrons need not necessarily go into the same kind of orbit in order to be symmetrically related. For example, in the ^1S state of the helium atom with one $1s$ and one $2s$ electron ($1s\ 2s$, ^1S), the two electrons are symmetrically related. When two like univalent atoms, e.g. two hydrogen atoms or two sodium atoms, unite to form a stable molecule, the two valence electrons become symmetrically related and are undoubtedly both in the same kind of orbit. But when two unequal atoms, e.g. sodium and hydrogen, unite, although their valence electrons become symmetrically related, it is open to some question whether they should be considered as being in the same kind of orbit (cf. discussion of heavier diatomic molecules on p. 379).

symmetrical relation *per se*, since it gives a positive contribution to the total energy, tends to weaken the binding of the two atoms.

Two helium atoms

The case of two helium atoms is similar to that of two hydrogen atoms. In the latter case, however, there was a choice between the two molecular electron configurations $(1s\sigma)^2$, which is ordinary stable H_2, and contains one valence bond according to usual valence theory, and $1s\sigma\, 2p\sigma$ where there is no valence bond. But in the helium case, there is no choice. Each helium atom has two $1s$ electrons to begin with, and if the atoms came together this would make four $1s$ electrons. But we know that no atom can have more than two $1s$ electrons, and this holds equally for a molecule. (This is an example of what is known as the Pauli exclusion principle.) Hence when the two helium atoms approach each other, two of the $1s$ electrons necessarily begin to be promoted to $2p\sigma$ electrons. From our empirical knowledge of the properties of helium gas, it is evident that these two anti-bonding electrons overpower the effect of the two bonding $1s\sigma$ electrons, causing the atoms to repel each other even at fairly large distances, so that no molecule is formed. Calculations by the method of Heitler and London give the same result (7).

London and Heitler's spin theory of valence

The principles that underlie these well-understood results for hydrogen and helium are presumably the same that must be used for explaining valence in general. London and Heitler tried to generalize the results of their calculations on these atoms in their well-known spin theory of valence (8). They noticed that in hydrogen a valence bond is formed when two originally unpaired electrons become paired and both go into the same kind of orbit, i.e., when they become symmetrically related.[6] London and Heitler then *postulated* that this establishment of a symmetrical relation between two electrons originally belonging to two separate atoms is characteristic of valence bonds in general. Thus they had a quantum-mechanical theory closely paralleling the Lewis theory of bonding electron pairs (9), at least for the usual case where each atom contributes one member of the pair.

In their theory, the valency, V, of an atom is supposed to be equal to the number of unpaired electrons. Since each unpaired electron has a spin quantum number $\frac{1}{2}$, and since all these unpaired spins stand parallel to each other, they give a resultant spin quantum number S for the atom which is equal to $V/2$. Thus it happens that in Heitler and London's theory the spin S can be taken as a convenient indicator of the number of free valences: $V = 2S$. The resultant spin in turn is related to the "multiplicity" m: $m = 2S + 1$. It also happens, in consequence of the Pauli principle, that when unpaired electrons become paired, i.e., symmetrically related, in a molecule, their spins pair off too, the members of each pair being arranged with their axes opposite so as to make a zero contribution to the resultant spin. If two atoms, each having the same number n of unpaired electrons and a spin $S = n/2$, unite in such a way that all the electrons thereby become paired off, the molecule has a spin of zero, and, incidentally, is as a result diamagnetic in all ordinary cases. This corresponds, according to London and Heitler, to the formation of a multiple valence bond, e.g., if $n = 3$, a triple bond.

London and Heitler's theory is enticingly simple but, in the opinion of the author, really does not hit the nail on the head. For many types of atoms, to be sure, especially if at least one atom is in an S state, it gives nearly always the correct valence, although in some cases it is necessary to make use of atoms in somewhat excited states. In other cases, however, especially atoms in P, D, and other states, it does not work so well. Moreover, its emphasis on spins and the pairing of spins, or even on the pairing of electrons, i.e., the establishment of symmetrical relations between them, seems misleading to the author, for reasons that have been outlined in a preceding section entitled "Application of Heitler and London method. . . . ."[7] The presence of unpaired electrons and their spins, and their pairing in molecules, undoubtedly act usually as convenient indicators

[7] Kemble and Zener (Phys. Rev. **33**, 512 (1929)) and Urey (Ruark and Urey: Atoms, Molecules, and Quanta, p. 687. McGraw-Hill Book Company, New York (1930)) also conclude that the symmetric relation of the electrons *per se* is relatively unimportant for molecular stability. See also Herzberg (footnote 1) and others.

of valence and of the formation of valence bonds, respectively, but even then in the author's opinion, they conceal something which is more fundamental.

This something is clearly revealed even in the simple example of the formation of the hydrogen molecule. Here a stable molecule is formed simply because $1s$ orbits in hydrogen atoms are capable of being sufficiently more firmly bound when they have two hydrogen nuclei to run around than when each has only one. The fact that two electrons become paired, i.e., that one comes from each atom and that they then become symmetrically related, seems to be largely incidental, as does also the pairing of the spins. In H_2^+, where there is only one electron, the case is even clearer, since here there is no possibility that pairing of electrons can be of importance for the result. What is fundamental here, it seems to the author, is that we have in the $1s$ orbit in hydrogen an orbit whose character permits it to become considerably more firmly bound when the hydrogen atom unites with another hydrogen atom, or in fact with any other kind of atom which can accept this electron without the necessity of an excessive promotion energy.

The molecules HeH, He₂⁺, CaH, and so on

In seeking further light on valence theory, one may ask, why should not a helium atom, with two $1s$ electrons, be able to unite with a hydrogen atom to form a stable molecule? Calculations by the method of Heitler and London show that these two atoms do *not* form a stable molecule (10). The question is one that the valence theory of London and Heitler accounts for very promptly by saying that the two $1s$ electrons in helium are already paired, their valences being mutually satisfied inside the helium atom, so that the latter is incapable of forming a compound with any other atom. From the present point of view, however, the explanation is different. It is known from the Pauli principle that no atom or molecule can contain more than two $1s$ electrons, at least not if these $1s$ orbits get near one another. Hence the $1s$ electron of the hydrogen atom, when it comes into contact with a

helium atom containing two $1s$ electrons, is promoted to a $2p\sigma$ orbit. It is easy to understand that this can take so much energy that the two atoms repel each other.

But in other analogous cases, it is known from spectroscopic evidence that a physically stable molecule is formed. Thus a helium atom (two $1s$ electrons) and a helium ion (one $1s$ electron) unite to form a fairly stable molecule $He_2^+(1s\sigma^2\, 2p\sigma)$. Here the two bonding electrons $1s\sigma$ evidently outweigh the one anti-bonding electron (this idea is taken from Herzberg's paper (see footnote 1).

There seems to be no good reason to suppose that the difference in behavior of $He + H$ and $He + He^+$ is other than a quantitative one. Possibly, to be sure, the fact that one of the particles which unite to form He_2^+ is an ion is partly responsible for the difference. But it is known from band spectrum evidence that such neutral molecules as MgH and CaH are formed with heats of dissociation of 30,000 calories or more, from unexcited magnesium or calcium atoms, whose electrons are all paired like the electrons in helium. Other examples can also be cited in which atoms with $S = 0$, hence $V = 0$ according to London and Heitler, form stable molecules. Secondary valence compounds, such as for example the $Cu(NH_3)_4^{++}$ ion, which are ascribed by Lewis and others to the bonding action of already formed electron pairs in the NH_3 molecules (9), also fail to come within the theory of Heitler and London.

Orbital valence

Heitler has sought to supplement the theory of spin valence by introducing the concept of "orbital valence." The idea is that in the case of atoms in $P, D, \ldots$ states, certain changes occur in the electron orbits (more precisely, in the "coupling of the l vectors"), giving rise to valence bonds (8). This idea when applied seems, however, to involve nothing different than is obtained by considering the effects of the molecular electric axis or axes in removing the degeneracy of the molecular electron orbits with $l > 0$ ($p, d, \ldots$ orbits) (11). Pauling and Slater have shown with striking success how such effects may suffice to

account for a great many of the directional and other related properties of chemical bonds (12).

Perhaps it will be best at this point to leave the perplexing problem of trying to find a general quantum-mechanical theory of non-polar valence. The author hopes to take up the discussion of this problem again in a later paper.

III. VALENCE IN SIMPLE HYDRIDE MOLECULES

Carbon-hydrogen compounds

Meantime it may be profitable, in the present review, to try to understand, by a somewhat detailed consideration of electron orbits, the relations between a number of typical molecules and their atoms. The examples already considered, in particular the diatomic molecules of hydrogen and helium, have yielded the concepts of bonding and anti-bonding electrons, of promotion, and of the effect of the Pauli principle in requiring promotion of any electron which would otherwise be in an orbit occupied by two other electrons. The importance of all these concepts becomes increasingly evident in a study of more complicated molecules.

First let us consider the step-by-step building-up of a methane molecule from a carbon atom and four hydrogen atoms. An equation for the formation of a CH molecule can be written as follows:

$$C(1s^2\ 2s^2\ 2p^2) + H(1s) \rightarrow CH(1s^2\ 2s^2\ 2p^3) + D',$$

or, more accurately,

$$C(1s^2\ 2s^2\ 2p^2, {}^3P) + H(1s, {}^2S) \rightarrow CH(1s\sigma^2\ 2s\sigma^2\ 2p\sigma^2\ 2p\pi, {}^2\Pi) + D',$$

Here D' stands for the heat of dissociation of CH into $C + H$. The symbols in parentheses describe the electron configurations and electron states of the atoms and molecule. Thus the symbols for the carbon atom mean that it contains two $1s$, two $2s$, and two $2p$ electrons (this is its electron configuration) and, less

important, that the atom as a whole is in what is called a ^3P (triplet P) state. The hydrogen atom contains one $1s$ electron, and is in a ^2S state. In the carbon atom, there are only two of the very firmly bound $1s$ electrons, because according to the Pauli exclusion principle, more than two are not allowed in one atom. The $1s$ electrons here are very much more firmly bound than the $1s$ electron of the hydrogen atom, because of the much greater charge on the carbon nucleus. As with the $1s$, there are only two of the next most firmly bound kind of electrons, namely $2s$. The groups $1s^2$ and $2s^2$ can be called closed groups or closed shells, since no more electrons can be admitted. The last two electrons are $2p$, which comes next in order of binding.

In the symbols describing the CH molecule, there are again only two $1s$ and two $2s$ electrons (denoted above by $1s\sigma^2$ and $2s\sigma^2$), in accordance with the Pauli principle. There are, however, three $2p$ electrons. This is not in conflict with the Pauli principle, because p electrons, unlike s electrons, can appear in more than one form. So long as not more than two orbits of the same form are present, the Pauli principle is satisfied. In the example of CH, two of the $2p$ electrons are in the form $2p\sigma$, giving a closed shell, and one is in the form $2p\pi$.[4] On looking into what has happened in the formation of the molecule, it appears that one of the $2p\sigma$ electrons in CH is nothing other than the $1s$ hydrogen electron, promoted, while the other $2p\sigma$ and the $2p\pi$ electron are nothing other than the two $2p$ electrons of the carbon atom (4).

In the case of a single atom, the distinction between $2p\sigma$ and $2p\pi$ electrons is not made. It comes into existence only when an axis of electrical (or magnetic) force is set up, such as is produced here by the charge of the hydrogen nucleus.

In the formation of the CH molecule, the most obvious change in the electron orbits of the atoms is that the $1s$ electron of the hydrogen has been promoted to $2p\sigma$ and has joined the ranks of the carbon electrons. In a previous example, that of two hydrogen or two helium atoms, promotion of a $1s$ electron to $2p\sigma$ caused the atoms to repel each other. In the present case, however, it does not prevent the atoms from attracting each other

strongly, giving a stable molecule with a heat of dissociation of the order of 90,000 calories, according to band spectrum data. Why is this? It is essentially because in spite of the promotion from $1s$ in hydrogen to $2p\sigma$ in CH, the firmness of binding of the electron is not decreased, because of the relatively high effective charge of the carbon nucleus. In general, the energy of binding of an orbit of given type varies as the square of the effective nuclear charge. We may assume as a reasonable guess that the promoted $1s$ electron is just as firmly bound in CH as in H. This alone, to be sure, would not suffice to give a stable CH molecule. But there can be little doubt that the electrons originally on the carbon atom become on the whole more firmly bound in the presence of the hydrogen nucleus, and that the sum total of energy changes suffices to account for the stability of the molecule. The increases in binding energy of the carbon electrons may be thought of as resulting from polarization of their orbits by the hydrogen nucleus.

In discussing the formation of a CH molecule, it has been assumed that the molecule is in its most stable, or normal, state. There are really four ways in which a carbon and a hydrogen atom could come together, one leading to repulsion, as in the case of the second mode of interaction of two hydrogen atoms considered above (12a). But here and in what follows we are interested only in the most stable molecular state which can be obtained from two given atoms, and from now on only this will be dealt with, although in most cases there are also other modes of interaction, which are important in spectroscopic and other problems.

The CH molecule has the same number of electrons as the nitrogen atom, and is indeed identical in its electron configuration with what would be obtained if one could detach a proton from a nitrogen nucleus in a nitrogen atom[8] and let the system come to equilibrium with a minimum of disturbance. This close relationship to the nitrogen atom will be made use of later.

Meantime let us consider the series of molecules CH_2, CH_3,

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[8] Strictly speaking, one must start with a slightly excited (^2D) nitrogen atom.

CH$_4$. Without going into details as to the subdivision of the p orbits into different forms,[9] we can write

$$CH(1s^2 \, 2s^2 \, 2p^3) + H(1s) \rightarrow CH_2(1s^2 \, 2s^2 \, 2p^4) + D''$$

$$CH_2(1s^2 \, 2s^2 \, 2p^4) + H(1s) \rightarrow CH_3(1s^2 \, 2s^2 \, 2p^5) + D'''$$

$$CH_3(1s^2 \, 2s^2 \, 2p^5) + H(1s) \rightarrow CH_4(1s^2 \, 2s^2 \, 2p^6) + D''''$$

The molecules CH$_2$, CH$_3$, and CH$_4$ have respectively the same electron configurations as the oxygen, fluorine and neon atoms. Of course all the orbits are more or less deformed, and the p orbits become differentiated into not more than three sub-forms, as a result of the fact that parts of the positive charge are located in the protons instead of being concentrated in the central nucleus. Or probably, as Pauling and Slater have shown (12), all the outer orbits are so much modified that we no longer should distinguish $2s$ and $2p$ orbits, but may better think, in CH$_4$, in terms of four new 2-quantum orbit-types, each a sort of hybrid of $2s$ and $2p$, with $2p$ predominating in the mixture. These new orbit-types are adapted to the probable tetrahedral symmetry of the molecule. In CH$_4$ and other polyatomic molecules, a considerable part of the energy of formation probably results from the possibility for the geometrical configuration of the nuclei and the orbits of the valence electrons to adjust themselves mutually to give minimum energy.

As each hydrogen atom is added, its $1s$ electron is promoted to a 2-quantum orbit. This goes on, giving a stable molecule at each step, so long as the maximum number of 2-quantum orbits allowed by the Pauli exclusion principle, namely eight (two $2s$ and six $2p$, or eight tetrahedral-type), is not exceeded. When this maximum number is reached, as in methane, the molecule strongly resembles the isoelectronic atom neon in having a very low boiling point and in refusing to combine stably with other atoms or

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[9] In molecules composed of more than two atoms, the nature of these orbit-types is usually different from that of those in diatomic molecules, and less easy to explain. It is still true, however, that s electrons give only one type; p electrons give not more than three types. Or, if the molecule is sufficiently stable, a hybridization of s and p electrons may occur. The total number of orbit-types derived from s and p together is not more than four in any case.

molecules. Methane is physically less inert than neon because parts of its positive charge, namely the four protons, are near the surface. Chemically also it is less inert, because it is not very difficult to remove one or more of these protons (together with an equal number of electrons), whereas in neon it would be very difficult to remove a proton from the nucleus.

Why is methane as inert as it is, and why does it not take on more hydrogen atoms to form CH_5, CH_6, and so on? The interpretation in terms of the principles here used seems to be clear. If one should bring a CH_4 molecule and a hydrogen atom together, the hydrogen $1s$ electron could no longer be promoted merely to a 2-quantum orbit but would have to go up to at least the next higher stage of promotion, which is a $3s$ orbit. Now from our knowledge of the spectra of the neon and sodium atoms, we know that a $3s$ orbit in sodium is bound only about one-fourth as firmly as the last $2p$ orbit in neon. Roughly the same relation probably would hold between a $3s$ and the last 2-quantum orbit in CH_5 if this molecule were stable. Hence although we may write

$$CH_4(1s^2\,2s^2\,2p^6) + H(1s) \rightarrow CH_5(1s^2\,2s^2\,2p^6\,3s)$$

we cannot expect CH_5 to be even a physically stable molecule. Possibly it has a slight physical stability, but it is certainly not chemically stable. The energy required to promote the hydrogen $1s$ electron to $3s$ is so large that the hydrogen atom is probably repelled by the CH_4 molecule, except perhaps at large distances. The $3s$ orbit thus acts as an anti-bonding one. The same difficulties would be met in trying to form a molecule, e.g., CH_6, with more hydrogen atoms. Urey[1] has also explained the non-existence of molecules like CH_5 in the same way as that given here.

We must next explain why the molecules CH, CH_2, CH_3, although stable as individuals, are not chemically stable. A sufficient explanation follows from the fact, already noted, that these molecules have essentially the same electron configurations as the atoms nitrogen, oxygen, and fluorine. According to the quantum theory, the behavior of two neutral atoms on coming together depends primarily on the nature and number of their electron

orbits. The same should be true of radicals such as CH, CH_2, and CH_3. We may therefore say that if we can explain why nitrogen, oxygen, and fluorine form stable molecules N_2, O_2, F_2, then we understand why C_2H_2, C_2H_4, and C_2H_6 are stable. N_2, O_2, F_2, and C_2H_4 will be considered a little later.

Boron-hydrogen compounds

The same methods that have just been applied to the compounds of carbon with hydrogen can also be applied to other hydrides. In the case of boron, for example, we might expect BH, BH_2, BH_3, BH_4, BH_5 all to be capable of stable existence as individual molecules. We know from spectroscopic work that this is true of BH. The fact that BH, BH_2, and BH_3 are not found as chemically stable individuals is not surprising, since their electron configurations must be the same as those of the atoms carbon, nitrogen, and oxygen. The existence of B_2H_6, for example, although contrary to ordinary valence theory, is explained in the same way as that of O_2. (See p. 381 for further details.) This idea is not a new one, but it is worthy of note that the present method gives it a very simple and obvious justification.[10]

But what about BH_5, whose existence as a gas resembling CH_4 seems to be predicted by the present method? Its absence can very likely be explained by the following equations:

$$BH_3 + H \rightarrow BH_4; \quad BH_4 + H \rightarrow (BH_5 \rightarrow) BH_3 + H_2$$

In other words, although BH_4, as well as BH_3, is doubtless capable of attracting another hydrogen atom rather strongly, the resulting molecule has probably a higher energy content than the system which results when a hydrogen molecule is split off. One of the main reasons why BH_5 should be less stable in this respect than CH_4 is that because of the smaller nuclear charge of the boron atom, the 2-quantum electrons in BH_5 are all decidedly less firmly bound than in CH_4. ·

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[10] Herzberg in his new paper has independently given the same explanation of B_2H_6 (footnote 1). It seems to the author that this is far simpler than one recently given by Pauling (14).

Polar molecules

It will be instructive at this point to consider the HF molecule as an illustration of the impossibility of drawing a sharp line between the polar and the non-polar bond. So far as the general principles of the quantum theory are concerned, either of the two following reactions might lead to identically the same result.

$$H(1s) + F(1s^2\ 2s^2\ 2p^5) \rightarrow HF(1s^2\ 2s^2\ 2p^6) + D$$

$$H^+ + F^-(1s^2\ 2s^2\ 2p^6) \rightarrow HF(1s^2\ 2s^2\ 2p^6) + D'$$

Whichever is correct, we may be sure that the HF molecule has the same electron configuration as the neon atom, although it is much less inert than the latter because of the fact that its positive charge is divided into two parts. It is not definitely known which of the above equations is the correct one. Very likely HF is formed from $H^+ + F^-$, while HCl is formed from $H + Cl$. But the properties of the finished molecule depend very little on the starting point and much more on the resulting electron configuration. A molecule having very little polarity could conceivably, according to the quantum theory, be formed from two ions, and a highly polar molecule conceivably from two neutral atoms. The HF molecule does indeed act like a dipole, because there is a slight excess of negative charge around the fluorine nucleus, although nothing like the excess of one whole electron which such a symbol as H^+F^- would suggest. Only in extreme cases, e.g., perhaps Cs^+F^-, is it really justifiable to think of a diatomic vapor molecule as actually even approximately *consisting of* two ions. A molecule like HF or HCl may correctly be said to be *formed from* two ions, or from two atoms, as the case may be, but what it really consists of may better be thought of as a unitary electron configuration whose outer shell surrounds both nuclei. The inner electrons, of course, remain close to the fluorine or chlorine nucleus.

Similar considerations apply to polyatomic molecules. For example, H_2O might conceivably be formed from $O + H + H$, or from $O^- + H^+ + H$, or from $O^- + H^+ + H^+$, but in any case the finished molecule would be essentially an electron configura-

tion $2s^2\ 2p^6$ surrounding the two hydrogen nuclei and the oxygen nucleus with its two $1s$ electrons. Actually, the stable (normal) state of H_2O, although strongly polar like HF, is probably formed from neutral atoms $O + H + H$. Again, CH_4 might conceivably be reached by the route $C^= + 4H^+$, or from $C + 4H$ as we have assumed, or in other ways, but our understanding of the nature of the CH_4 molecule does not depend on a knowledge of how it might be formed from atoms or ions.

Superfluity of the concept of valence bonds in the "molecular" point of view

In the "molecular" point of view advanced here, the existence of the molecule as a distinct individual built up of nuclei and electrons is emphasized, whereas according to the usual atomic point of view the molecule is regarded as composed of atoms or of ions held together by valence bonds. From the molecular point of view, it is a matter of secondary importance to determine through what intermediate mechanism (union of atoms or ions) the finished molecule is most conveniently reached. It is really not necessary to think of valence bonds as existing in the molecule. In hydrides, it is perhaps better not to think in terms of valence bonds between the hydrogen and other atoms, although in the case of heavier atoms, it is usually more convenient to assume valence bonds between them. (For an instructive example involving both cases, compare the discussion of B_2H_6 and C_2H_4 at the end of the section on "Polyatomic hydrides with two heavy atoms," on p. 380.) But whenever one is in doubt as to what kind of binding is present, e.g., polar or non-polar, the molecular point of view serves as the natural means to reconciliation.

IV. VALENCE IN COMPOUNDS CONTAINING TWO MANY-ELECTRON ATOMS

In the most stable molecules formed from two atoms, each containing several or many electrons (e.g., N_2, CO), the outer shells are often rather thoroughly shared, and are properly thought of for many purposes as forming a single unit. In less stable molecules, e.g., the halogens, the outer shells are only incompletely

shared, and the molecule may often be thought of as consisting of two atoms. Just what these statements mean can best be seen by considering again a series of examples.

Electron configurations of atoms and molecules

The results will be given first and the explanation afterwards. The electron states and probable electron configurations of several diatomic molecules formed from such atoms as lithium, nitrogen, oxygen, and fluorine are essentially as given in the following:

$2Li \ [(1s)^2 2s, \ ^2S] \rightarrow Li_2 \ [(1s)^2 \ (1s)^2 \ (\sigma 2s)^2, \ ^1\Sigma_g^+]$

$N \ [(1s)^2 \ (2s)^2 \ (2p)^3, \ ^4S] + C \ [(1s)^2 \ (2s)^2 \ (2p)^2, \ ^1P]$

$\rightarrow CN \ [(1s)^2 \ (1s)^2 \ (\sigma 2s)^2 \ (\sigma^*2s)^2 \ (\pi 2p)^4 \ \sigma 2p, \ ^2\Sigma^+]$

$2N \ [(1s)^2 \ (2s)^2 \ (2p)^3, \ ^4S] \rightarrow N_2 \ [(1s)^2 \ (1s)^2 \ (\sigma 2s)^2 \ (\sigma^*2s)^2 \ (\pi 2p)^4 \ (\sigma 2p)^2, \ ^1\Sigma_g^+]$

$O \ [(1s)^2 \ (2s)^2 \ (2p)^4, \ ^3P] + C \ [(1s)^2 \ (2s)^2 \ (2p)^2, \ ^1P]$

$\rightarrow CO \ [(1s)^2 \ (1s)^2 \ (\sigma 2s)^2 \ (\sigma^*2s)^2 \ (\pi 2p)^4 \ (\sigma 2p)^2, \ ^1\Sigma^+]$

$N \ [\ldots (2p)^3, \ ^4S] + O \ [\ldots (2p)^4, \ ^3P] \rightarrow NO \ [\ldots (\sigma 2p)^2 \ (\pi 2p)^4 \ \pi^*2p, \ ^2\Pi]$

$2O \ [\ldots (2p)^4, \ ^3P] \rightarrow O_2 \ [\ldots \ (\sigma 2p)^2 \ (\pi 2p)^4 \ (\pi^*2p)^2, \ ^3\Sigma_g^-]$

$2F \ [\ldots (2p)^5, \ ^2P] \rightarrow F_2 \ [\ldots (\sigma 2p)^2 \ (\pi 2p)^4 \ (\pi^*2p)^4, \ ^1\Sigma_g^+]$

$2Na \ [(1s)^2 \ (2s)^2 \ (2p)^6 \ 3s, \ ^2S] \rightarrow Na_2 \ [(Na^+)_2 \ (\sigma 3s)^2, \ ^1\Sigma_g^+]$

Nomenclature and bonding properties of orbit-types

The designations $\sigma 2s$, σ^*2s indicate σ electrons derived from atomic $2s$ electrons. Similarly $\sigma 2p$, $\pi 2p$, π^*2p indicate σ or π electrons derived from $2p$ atomic electrons.[4] Designations of this sort, first used by Lennard-Jones (who, however, used the symbols $2s\sigma$, $2s\sigma'$, $2p\sigma$, and so on for this purpose) are more appropriate for most molecules, except hydrides, than designations like $2s\sigma$, $2p\sigma$ so far used in this review.[11] The latter sym-

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[11] Instead of $\sigma 2s$, σ^*2s, $\sigma 2p$ it would be more accurate in some cases to use designations such as $z\sigma$, $y\sigma$, $x\sigma$ which do not imply definite relationships to $2s$ and $2p$ atomic orbits (see reference 4 for details). This is because in some of the molecules here under consideration there must be a partial breakdown of the s-p distinction, such as Slater and Pauling (12) assume.

Pauling in his discussion of the molecules CO, CN, N_2, and NO (reference 12, pp. 1383–5), uses the symbols σ_0 and σ_b, the first corresponding to $\sigma 2s$ and σ^*2s (or

bols are based on a consideration of what the orbits designated would become if the distance between the nuclei could be steadily decreased to zero, giving the "united-atom." Except in hydrides, however, the electron orbits are much more closely related to those of the separate atoms to which they originally belonged.[12]

It is not possible to give a completely unambiguous set of correlations between the separated-atoms and the united-atom designations for molecular orbits. The following, however, are the usual correlations:

$$\sigma 1s \rightarrow 1s\sigma; \; \sigma^*1s \rightarrow 2p\sigma \text{ or } 2s\sigma;^{18} \; \sigma 2s \rightarrow 2s\sigma \text{ or } 2p\sigma; \; \sigma^*2s \rightarrow 3p\sigma \text{ or}$$

$$3s\sigma; \; \sigma 2p \rightarrow 3s\sigma \text{ or } 3p\sigma; \; \pi 2p \rightarrow 2p\pi; \; \pi^*2p \rightarrow 3d\pi \text{ or } 3p\pi.$$

It will be seen that with the exception of $\sigma 1s$, $\sigma 2s$, and $\pi 2p$, all the orbits named become promoted orbits in the united-atom. For the most part, outer-shell electrons in unpromoted orbits are bonding, while those in promoted orbits are anti-bonding (13), especially in molecules whose atoms are in the same or in not far distant columns of the periodic system. Exceptions will be discussed shortly. More accurately—as it *happens* (?)—, the unstarred orbit-types always correspond to bonding, the starred types (e.g., σ^*2s, π^*2p) to anti-bonding electrons, in molecules composed of like or not too unlike atoms.

Li₂ molecule: non-bonding inner electrons

The relations between atomic and molecular electron configurations given above can be interpreted in terms of bonding and

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$z\sigma$ and $y\sigma$) and the second to $\sigma 2p$ (or $x\sigma$). The author finds himself to a considerable extent unable to agree with Pauling's treatment of these molecules, which he thinks suffers from the failure to distinguish between $\sigma 2s$ and σ^*2s and between $\pi 2p$ and π^*2p orbit-types, and also from the assumption that the pairing of two originally unpaired electrons is usually necessary for the formation of a valence bond. Pauling considers O_2, however, and in a later paper (14) also NO, to be partial exceptions to this pairing rule.

[12] In a previous article (Chem. Reviews **6**, 532 (1929)) these united-atom designations have been used throughout.

[18] The first four electrons in the electron configuration of each of the molecules given above might have been designated $(\sigma 1s)^2(\sigma^*1s)^2$, but for reasons to be given shortly, the simpler symbols $(1s)^2(1s)^2$ have been used.

anti-bonding electrons, etc., as follows.   When two lithium atoms approach, their $2s$ electrons become more firmly bound and move in the field of both nuclei, just as the $1s$ electrons of two hydrogen atoms do in the formation of the hydrogen molecule.   The two $\sigma 2s$ electrons in Li_2 can be called bonding electrons, and they may be said to form a valence bond.   The energy of formation of Li_2 is, however, much less than that of H_2, mainly because the $2s$ electrons are much less firmly bound in Li and in Li_2 than are the $1s$ electrons in H and H_2.[14]   Since the total binding energy is so much less, the change in binding energy when the molecule is formed is naturally also less, in somewhat the same proportion.

The two $1s^2$ shells of the two atoms tend to cause the latter to repel each other and, technically, two of the $1s$ electrons begin to be promoted to $\sigma^* 1s$ orbits, just as when two helium atoms approach.   But the distance between the nuclei in Li_2 is so large, namely 2.67×10^{-8} cm., when equilibrium is reached under the bonding action of the two $\sigma 2s$ electrons, that the promotion energy of the two $1s^2$ shells is negligible.   This is because the $1s^2$ shells in the lithium atoms are only about 0.2×10^{-8} cm. in radius, and because the "exchange" forces between two such groups of electrons are not large if the distance between them much exceeds their own dimensions.   The $1s$ electrons in Li_2 may evidently appropriately be called non-bonding electrons.   This is indicated in the configuration formula given for Li_2 by the use of the simple designations $(1s)^2 (1s)^2$.   The same is true of the $1s$ electrons in all molecules composed of atoms each containing several electrons.   In fact, in the case of molecules composed of atoms containing many electrons, all inner closed shells of the atoms act, like the $1s$ electrons in Li_2 and for the same reasons, as non-bonding electrons (cf. the inner electrons in Na_2).   Only outer electrons take an active part in the formation of molecules.

[14] The values of the energy of formation of Li_2 and of Na_2 have been calculated by the Heitler and London method.   The results agree very well with the observed values.   For Li_2 see DELBRÜCK, M.: Ann. der Physik [5] **5**, 36 (1930) and BARTLETT, J. H., JR. AND FURRY, W. H.: Phys. Rev. **37**, 1712 (1931).   For Na_2 see ROSEN, N.: Phys. Rev. **38**, 274 (1931).

Nitrogen molecule (also CO, CN)

In the case of the nitrogen molecule, there are five outer electrons in each of the atoms from which it is formed. Of the four $2s$ electrons in the two atoms, two become $\sigma 2s$ electrons (unpromoted type) while two become $\sigma^* 2s$ (promoted type). The spectroscopic facts about the N_2 molecule indicate (cf. table 1) that there is a relatively very large decrease (perhaps 150,000 calories per electron) in firmness of binding of the atomic $2s$ electrons when they become molecular $\sigma^* 2s$ electrons. But the anti-bonding action indicated by this is presumably approximately balanced, although we have no spectroscopic evidence thereof, by a strong bonding action on the part of the $\sigma 2s$ electrons, so that the group $(\sigma 2s)^2 (\sigma^* 2s)^2$ as a whole has very likely nearly a non-bonding action, or a mild anti-bonding action.

Next come four $\pi 2p$ and two $\sigma 2p$ electrons, all bound with about equal firmness in the molecule, as is shown by the band spectra of nitrogen. These electrons are all considerably more firmly bound in the molecule than in the atoms, as is shown by spectroscopic facts (see table 1) and must therefore all be classed as bonding electrons. If we now count up all the electrons in the molecule, there are two pairs of non-bonding electrons $(1s)^2(1s)^2$, four pairs of bonding electrons $(\sigma 2s)^2$, $(\pi 2p)^4$, $(\sigma 2p)^2$, and one pair of anti-bonding electrons $(\sigma^* 2s)^2$. The practical result is about the same as if there were just three bonding pairs $(\pi 2p)^4$, $(\sigma 2p)^2$ with all the other electrons acting as non-bonding, and in this sense we may say that there are three valence bonds between the atoms. The discussion which has just been given shows, however, that the real state of affairs is less simple in that the $\sigma 2s$ and $\sigma^* 2s$ electrons are really far from being unshared and non-bonding as the $1s$ electrons are.

The $\sigma 2p$ orbit-type is of special interest in that it acts as a bonding type in spite of its promoted character ($\sigma 2p \rightarrow 3s\sigma$ or $3p\sigma$ in the united-atom). According to Pauling (14), $\sigma 2p$ should give a stronger bond than $\pi 2p$. Actually, however, the bonding powers of $\sigma 2p$ and $\pi 2p$ are about equal in N_2, while in CN and CO the bonding power of $\sigma 2p$ appears to be less than that of

TABLE 1

Ionizing potentials of molecules

MOLECULE	STATE	ELECTRON IONIZED	STATE OF ION	IONIZATION POTENTIAL[1]	IONIZATION POTENTIAL, CORRECTED[2] FOR r	ATOMIC IONIZATION POTENTIAL CORRECTED[3]
H₂	$(1\sigma)^2$, $^1\Sigma_g{}^+$	1σ	$^2\Sigma_g{}^+$	15.4	15.9	13.54 (uncorrected)
CO	$(\sigma 2s)^2(\sigma^*2s)^2(\pi 2p)^4(\sigma 2p)^2$, $^1\Sigma^+$	$\sigma 2p$	$^2\Sigma^+$	14.2	14.2	10 (C)
		$\pi 2p$	$^2\pi$	16.7	17.3	14 (O)
		σ^*2s	$^2\Sigma^+$	19.8	19.8	20 (C)
N₂	$(\sigma 2s)^2(\sigma^*2s)^2(\pi 2p)^4(\sigma 2p)^2$, $^1\Sigma_g{}^+$	$\sigma 2p$	$^2\Sigma_g{}^+$	16	16	12
		$\pi 2p$	$^2\pi$	16 (?)	16.5 (?)	12
		σ^*2s	$^2\Sigma_u{}^+$	19	19	24
NO	$(\sigma 2p)^2(\pi 2p)^4(\pi^*2p)$, $^2\Pi$	π^*2p	$^1\Sigma^+$	9.4	9.9	14 (N)
O₂	$(\sigma 2p)^2(\pi 2p)^4(\pi^*2p)^2$, $^3\Sigma_g{}^-$	π^*2p	$^2\pi_g$	12.5	12.5	17
		$\pi 2p$	$^2\pi_u$	17.2	17.8	17
HCl	$(3p\sigma)^2(3p\pi)^4$, $^1\Sigma^+$	$3p\pi$	$^2\pi$	13.8	13.8	13 (Cl)

[1] The molecular ionization potentials given here represent the minimum values theoretically capable of producing ionization. Cf. H. D. Smyth, Rev. Modern Physics, **3**, 347 (1931), and R. S. Mulliken, Phys. Rev. **32**, 186 (1928), for data.

[2] The "corrected" molecular ionization potentials correspond to ionization of the molecule without change in the distance r between the nuclei.

[3] The atomic ionization potentials given here for comparison have been corrected to make a rough allowance for the change in multiplicity in the process of ionization. If, for instance, when the N_2 molecule is ionized a $^2\Sigma$ state, so that the multiplicity is increased from singlet to doublet, it is fairer to compare this with an atomic ionization process in which the multiplicity increases (e.g., $^3D \rightarrow {}^3P$ in the nitrogen atom) than with one in which it decreases (e.g., $^4S \rightarrow {}^3P$). But there seems to be no way of making accurate corrections so as to make these comparisons quantitative. In fact the significance of such comparisons is in general decidedly dubious from a theoretical standpoint, and their use for distinguishing bonding from anti-bonding electrons should be considered more or less empirical. The "corrected atomic ionization potentials" are based on a careful study of existing spectroscopic data. A long table of such data was at first included, but for the present purpose it did not seem worth the space required. See A. A. Noyes and A. O. Beckman, Chem. Reviews **5**, 85 (1928), for a condensed table, which, however, does not consider the various atomic states of different multiplicity which might be used in getting ionisation potentials.

$\pi 2p$. These statements are based on the relative ionization potentials of $\sigma 2p$ and $\pi 2p$ in CO and N_2 (cf. table 1).

It seems probable that in less stable molecules, where the distance r between the nuclei is larger, $\sigma 2p$ really has greater bonding power than $\pi 2p$, in agreement with Pauling. There is a little spectroscopic evidence for this (4). A reasonable explanation is the following. When r is large the $\sigma 2p$ and $\pi 2p$ orbits are essentially orbits of the original atom, modified by the electric field of the second atom. Because of the orientation of $\sigma 2p$ with reference to the electric axis, or better, because of the form of its wave-function, it is more strongly influenced than $\pi 2p$ by the second atom and so is more firmly bound. But when r is smaller, as in N_2 or CO at equilibrium, the effect of the promotion which must occur as $r \to 0$ causes the binding of $\sigma 2p$ to be decreased relative to $\pi 2p$, with the observed results. Both $\sigma 2p$ and $\pi 2p$ electrons, however, have high bonding power in N_2.

Molecules NO, O_2, F_2: anti-bonding $\pi^* 2p$ electrons

In the molecules NO, O_2 and F_2 the distance between the nuclei is greater than in N_2 and at the same time the $\sigma 2p$ orbits are smaller, since the $2p$ orbits of oxygen and fluorine are smaller than those of nitrogen. They should therefore be more firmly bound than the $\pi 2p$, and this has been assumed in writing the electron configurations of these molecules.

In the molecules NO, O_2, and F_2, we note a steady decrease in the heat of dissociation (cf. table 2), paralleling a steady increase in the number of $\pi^* 2p$ electrons assigned to their electron configurations. These $\pi^* 2p$ electrons are promoted electrons ($3d\pi$ or $3p\pi$ in the united-atom). They act strongly as anti-bonding electrons. This can be seen very clearly in a comparison of NO with N_2. In N + N, the lowest ionizing potential, corresponding to a removal of a $2p$ electron, is 14.5 volts. For removal of a $\sigma 2p$ or $\pi 2p$ electron from N_2, about 16 volts is required, indicating that these electrons are more firmly bound in N_2 than in N + N.[15]

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[15] One should not overemphasize the quantitative significance of such comparisons (cf. table 1, notes), but qualitatively they distinguish rather clearly between bonding and anti-bonding electrons.

For removal of a π^*2p electron from NO, only 9.4 volts are required, as compared with 14.5 volts for the corresponding $2p$ electron in the nitrogen atom of N + O, or of 13.6 volts if the

TABLE 2

Heats of dissociation (D) of some diatomic molecules†

MOLECULE	D	NUMBER OF BONDS (N)	D/N	MOLECULE	D	NUMBER OF BONDS (N)	D/N
	kg-cal.				kg-cal.		
H₂⁺.............	60.9	½	122	C₂.............	[126]	2	[63]
H₂.............	103	1	103	BO.............	[152]	2½	[61]
He₂⁺.............	[60]	½	[120]	CN.............	[164]	2½	[66]
HeH.............	0	½? or 0	—	N₂⁺.............	[157]	2½	[63]
HgH......... ..	8.5	½?	17?	CO⁺.............	(164)	2½	(66)
CaH.............	[35]	½? or 1?	[70?]	CO	(231)	3	(77)
LiH.............	57	1	57	N₂.............	[210]	3	[70]
CH.............	(92)	1	(92)	NO⁺.............	(238)	3	(79)
HF.............	148	1	57	NO.............	(142)	2½	(57)
HCl.............	102	1	(92)	O₂⁺.............	(143)	2½	(57)
Li₂.............	26	1	148	O₂.............	117	2	59
Na₂.............	18	1	102	S₂.............	102	2	51
			26	F₂.............	(66)	1	(66)
			18	Cl₂.............	57.0	1	57

† *(1)* The D values are all measured from the lowest energy level of the molecule, i.e., they are corrected to 0 K. *(2)* The values for H₂, Li₂, Na₂, O₂, Cl₂ are reliable values (probable error 0 to 2 kg-cal.) from band spectrum data, those for LiH, S₂, O₂⁺ are somewhat less reliable values from the same source. The value for HCl is based on reliable chemical data. Values for F₂ and CH are estimates which are probably rather accurate, that for He₂⁺ is an estimate of low reliability. Values for C₂, BO, CN, N₂⁺, N₂, are relatively unreliable values based on spectroscopic data or estimates from these. Values for CO, CO⁺, NO, NO⁺ are based on spectroscopic and chemical data combined. The value for H₂⁺ is based upon accurate quantum-mechanical calculations by Burrau and Hylleraas. Values in brackets are the least reliable, those in parentheses also not very reliable (probable error 5 to 15 kg-cal.); other values are rather reliable. All the values have been more or less critically considered, and some have been estimated or revised, by the author. *(3)* The number of bonds (N) is taken equal to half the difference between the numbers of bonding and anti-bonding electrons.

electron is derived from a $2p$ of the oxygen atom.[15] Clearly the π^*2p electron is much less firmly bound in NO than in N + O, and the corresponding strong anti-bonding action is responsible for the large decrease in heat of dissociation from N₂ to NO. As

we go to O_2 and F_2, each additional π^*2p electron causes a further decrease in the heat of dissociation, and in O_2 as in NO (no data are available for F_2) the ionizing potential of the molecule is considerably less than that of the atoms (table 1).

Transformation of bonding and anti-bonding electrons into non-bonding electrons with increasing atomic number and with decreasing valence

The reasons why two neon atoms refuse to form a molecule can now be at least qualitatively understood. But first it will be helpful to point out that in the series N_2, O_2, F_2 we not only have an increasing number of π^*2p electrons and a decreasing dissociation energy, but also a resulting increase in the equilibrium distance r_e between the nuclei. This has the values, 1.1, 1.2 and about 1.5, each times 10^{-8} cm., in N_2, O_2, and F_2. As a result of this increase in r_e, and of the concomitant decrease in size of the 2s and 2p orbits (due to increase in nuclear charge) in going from nitrogen to fluorine, the condition of the $\sigma2s$, σ^*2s, $\pi2p$ and $\sigma2p$ electrons must be very different in F_2 than in N_2. Probably in F_2 the strong bonding and antibonding effects which exist for the $\sigma2s$ and σ^*2s electrons in N_2 are very nearly gone, so that these electrons are in truth practically non-bonding electrons (15).

Roughly speaking, each $\sigma2s$ and σ^*2s electron in N_2 may be considered to belong almost equally to both nuclei, while in F_2 one electron of each kind probably belongs pretty definitely to each nucleus. More precisely (cf. discussion on p. 352), this means that in N_2, each $\sigma2s$ and σ^*2s electron moves back and forth from the vicinity of one nucleus to that of the other with about the same frequency as that of the orbital motion which the 2s electron in the nitrogen atom would have according to the Bohr theory, while in F_2 each $\sigma2s$ and σ^*2s electron moves very nearly as it would in a 2s orbit in a fluorine atom, and only occasionally, with a frequency very much less than that of the orbital motion, jumps from the neighborhood of one nucleus to that of the other, usually almost simultaneously with the transfer of another 2s electron in the opposite direction.

Now as for the $2p$ electrons of F + F or N + N, it is clear that they also must be much more nearly like atomic electrons in F_2 than in N_2. The differences, whether positive or negative, in firmness of binding in the molecule as compared with the atoms, must therefore be much less in F_2 than in N_2, for all the orbit-types derived from $2p$. The ionizing potential of F_2, for example, should be rather close to that of the fluorine atom. Also, the differences in binding energy between $\pi 2p$ and $\pi^* 2p$ should be much less in F_2 than in NO or N_2. Probably each of the $\pi 2p$ and of the $\pi^* 2p$ electrons in F_2 is more or less definitely attached to one nucleus, in the sense above described. Very likely the $\sigma 2p$ electrons, however, because of the form of their orbits and the probable firmness of their binding, are still pretty well shared by the nuclei. Probably one may with a considerable degree of truth think of the two $\sigma 2p$ electrons in F_2 as bonding electrons which constitute the single valence bond demanded by the ordinary rules of valence, and of all other electrons as non-bonding electrons. There cannot be much doubt, however, that in reality the $\pi 2p$ and $\pi^* 2p$ electrons are still acting, even though perhaps only rather weakly, as bonding and anti-bonding electrons respectively (15). It seems likely that in polyatomic molecules the situation commonly resembles that here described for F_2.

Two neon atoms

In Ne + Ne, we have two more $2p$ electrons than in F + F. Since F_2 contains four $\pi^* 2p$ electrons, which is the maximum number allowed by the Pauli principle, the two new $2p$ electrons must be promoted to some other kind of orbit. Moreover they must be even more loosely bound in this new kind of promoted orbit than are the $\pi^* 2p$ electrons, and must therefore exert an even stronger anti-bonding action. This we can be fairly sure of from the fact that the four least firmly bound electrons in ordinary F_2 are all $\pi^* 2p$. If any other *more* firmly bound orbit were available, some of the electrons would go into it.

On extrapolating from the heats of dissociation of N_2, O_2, and F_2, we see that the incorporation of two more strongly anti-bonding electrons, which we have just seen to be necessary in Ne_2,

must reduce the heat of dissociation to about zero. Thus we can understand why two neon atoms do not form a stable molecule. This understanding is of course semi-empirical in that it is partly based on a study of spectroscopic data.

On theoretical grounds and from the spectra of the halogen molecules, the last two electrons in Ne_2 are σ^*2p electrons, although it is not obvious just why this kind of orbit should have such a strong anti-bonding action. As in the case of He_2 (and of Be_2), the number of anti-bonding electrons is equal to the number of bonding electrons in Ne_2. Or, since the two neon atoms never come near enough together to get either strong bonding or strong anti-bonding reactions, we see that in Ne_2 all the electrons are non-bonding in fact. The same relations are true for any pair of (unexcited) atoms both composed only of closed shells. Such relations correspond to zero valence bonds.

Heavier diatomic molecules

Going on to $Na + Na$, we have a case very similar to that of $Li + Li$. The two $3s$ electrons of Na produce a weak binding of the two atoms which leads to equilibrium at such a large r_e that the repulsive "exchange" forces between the two neon-like inner shells are negligibly small.[14] In other words, we have again a clear-cut case of a pair of outer electrons forming a valence bond, while the inner electrons all act merely as non-bonding electrons.

The formation of molecules like SiO, ICl, LiNa can also be understood by this method. The reactions can be written with approximate correctness as follows (4):

$$Na\,[(K)(L)3s,\,^2S] + Li\,[(K)2s,\,^2S] \rightarrow NaLi\,[(K)(K)(L)(\sigma2s,\sigma3s),\,^1\Sigma^+]$$

$$Si\,[(K)(L)(3s)^2(3p)^2,\,^3P] + O\,[(K)(2s)^2(2p)^4,\,^3P]$$

$$\rightarrow SiO\,[(K)(K)(L)(\sigma2s)^2(\sigma^*3s)^2(\pi2p)^4(\sigma3p)^2,\,^1\Sigma^+]$$

$$I\,[(K)(L)(M)(N)(5s)^2(5p)^5,\,^2P] + Cl\,[(K)(L)(3s)^2(3p)^5,\,^2P]$$

$$\rightarrow ICl\,[(K)(L)(M)(K)(N)(L)(3s)^2(5s)^2(\sigma3p,\sigma5p)(\pi3p)^4(\pi^*5p)^4,\,^1\Sigma^+]$$

Here K, L, M, N stand for complete 1-, 2-, 3-, and 4-quantum shells. Pairs of symmetrically connected electrons like $(\sigma3p, \sigma5p)$ in ICl function as equivalent electrons in the molecule. If

one thinks in terms of separated-atom orbits, each electron must be considered to jump frequently from the I $(5p\sigma)$ to the Cl $(3p\sigma)$ orbit or vice versa. If one considers what happens as $r \to 0$ (united-atom), one concludes (probably) that the orbits of the two electrons become identical. When the electron configuration symbols are written as here, it is evident why molecules composed of atoms in the same columns of the periodic system (e.g., I_2, ICl, Cl_2, or CO, SiO, CS) are similar. Quantitative differences between similar molecules are, however, to be expected, since such differences exist in the atoms themselves.

The present method seems to offer a possibility of understanding such facts as that the resemblance between F_2 and Cl_2 is much closer than between N_2 and P_2. In the halogens, with their rather small heats of dissociation, the molecule is not much different from two atoms, so that the close resemblances between the atoms suffice to account for those between the molecules. In N_2, however, the electron orbits are so greatly modified by the strong bonding that the molecule has an individuality very distinct from that of its component atoms. P_2, on the other hand, has a different distinct individuality, or more probably it, as well as As_2, Sb_2, and Bi_2, acts more like a mere pair of atoms. In either case, its lack of close resemblance to N_2 is explained.

Polyatomic hydrides with two heavy atoms

As has been pointed out earlier, the formation of stable molecules C_2H_6, B_2H_6 and C_2H_4, C_2H_2 from the radicals CH_3, BH_3 and CH_2, and CH can be explained in the same way as the formation of F_2, O_2, and N_2 from fluorine, oxygen, and nitrogen atoms. Many other molecules, e.g., HCHO and N_2H_4, can be accounted for in a similar way.

The electron configuration of C_2H_2, as Herzberg· has pointed out,[1] can be described in exactly the same way as that of N_2. Being a linear molecule, so that the field of force in which its electrons move is symmetrical with reference to an axis, as in a diatomic molecule, its electrons can all be classified under the headings σ, π, ... just as in a diatomic molecule.

In a molecule like C_2H_4 the situation is somewhat altered.

Because the field of force in which the electrons move is no longer symmetrical around the line joining the carbon atoms, the orbit-types are now somewhat different. It is, however, not unreasonable to expect that the electron configuration of C_2H_4 is closely related to that of O_2, from which an isotope of C_2H_4 might be obtained by splitting off two hydrogen nuclei from each oxygen nucleus. Assuming ·the closest possible analogy, we have (cf. pp. 365, 370):

$O : (1s)^2(2s)^2(2p)^4, \, ^3P$

$O_2 : (1s)^2(1s)^2(\sigma 2s)^2(\sigma^*2s)^2(\sigma 2p)^2(\pi 2p)^4(\pi^*2p)^2, \, ^3\Sigma_g^-$

$CH_2 : (1s)^2(2sa)^2(2pb)^2(2pc)^2, \, ^1X$

$C_2H_4 : (1s)^2(1s)^2(a2s)^2(a^*2s)^2(b2p)^2(c2p)^2(d2p)^2(c^*2p)^2, \, ^1Z$

As was noted earlier (4), atomic p electrons split into two types σ and π in diatomic molecules (e.g., $2p\sigma$ and $2p\pi$ in CH), but in general in polyatomic molecules into three types. Of each of these, as of σ electrons in the diatomic case, it takes only two to make a closed shell. If the total number of electrons is even, and if all are in closed shells, as is probable for the stablest state, the molecule is necessarily in a singlet state ($S = 0$). In the case of the π electrons of a diatomic or linear polyatomic molecule, it takes four to make a closed shell. Two π electrons, plus any number of closed shells, give $^3\Sigma^-$ as the stablest state, as in O_2. The two bonding electron types $\sigma 2p$ and $\pi 2p$ of O_2 must probably be replaced in C_2H_4 by three which the author has called $b2p$, $c2p$, and $d2p$, while the anti-bonding types σ^*2p and π^*2p must probably also be replaced by three which may be called b^*2p, c^*2p, and d^*2p. It is assumed that c^*2p is the most firmly bound of these last and is therefore present in the normal state of C_2H_4. Two c^*2p electrons completing the electron configuration of C_2H_4 give a singlet state (which has been called ^1Z), in contrast to the $^3\Sigma^-$ normal state resulting from the two π^* electrons in O_2. This difference explains the fact that ethylene is diamagnetic while oxygen is paramagnetic.

A similar explanation of the electron configuration and diamagnetism of C_2H_4 has been given by Hückel (16), who, however,

has not considered carefully the existence of π^* as well as π (or c^* as well as c) electrons. Slater and Pauling (12) have given what at first sight seems an entirely different interpretation of the electronic structure of C_2H_4, in terms of atoms joined by valence bonds.

The present explanation of the electronic structures of C_2H_4, B_2H_6, and the like completely dispenses with the idea of valence bonds between hydrogen atoms and boron or carbon atoms (cf. "Superfluity of the concept of valence bonds" on p. 369). It regards the electrons originally belonging to the hydrogen atoms as having become integral parts of the electron systems of radicals such as CH_2 or BH_3 which then function much like ordinary atoms. The original hydrogen atoms are thought of as having each been resolved into an electron which behaves as just stated and a proton which finds an equilibrium position in the radical. When two radicals like CH_2 or BH_3 unite, their outer electrons, without distinction as to whether or not they originally belonged to hydrogen atoms, are assumed to act as bonding or anti-bonding electrons for the union of the two radicals. One can then, if one wishes, define the number of valence bonds between the two radicals as equal to half the difference between the numbers of bonding and anti-bonding electrons.

The truth probably lies somewhere between the present point of view which assumes complete loss of individuality of the hydrogen atoms in molecules, and that of ordinary valence theory as used by Slater and Pauling, which assumes a preservation of the individuality of each atom, including hydrogen atoms, in the molecule. The case here is similar to the fact that in most molecules the truth lies between the two extremes polar and non-polar. That hydrogen atoms may usually largely lose their identity in molecules while other atoms usually largely preserve theirs is not unreasonable, since the H^+ ion is uniquely small as compared with the ions of other atoms.

The success of the present method in accounting for the molecule B_2H_6 suggests that the present explanation of B_2H_6, and quite possibly also of C_2H_4, C_2H_2, and so on, is more nearly correct than are attempted explanations using ordinary valence

concepts. The author hopes to discuss these questions more fully elsewhere.

<p style="text-align:center">V. THE CHEMICAL BOND</p>

<p style="text-align:center">*Bonding energies in diatomic molecules*</p>

<p style="text-align:center">*What is the unit chemical bond?*</p>

If with Herzberg (3) one defines the number of valence bonds as equal to the number of pairs of bonding electrons minus the number of pairs of anti-bonding electrons, then Li_2, C_2, CN, N_2, CO, NO, O_2, and F_2 have respectively 1, 2, $2\frac{1}{2}$, 3, 3, $2\frac{1}{2}$, 2, and 1 valence bonds, in agreement, except for CN, CO, and NO, with ordinary valence theory. Using the same method of definition, the molecules H_2^+ and He_2^+ have each $\frac{1}{2}$ valence bond. Table 2 shows that when the number of bonds N is defined in this way, the heat of dissociation varies very little from a characteristic mean value for each value of N, in the molecules from C_2 to F_2. Further, the heat of dissociation per bond (D/N) is nearly constant. These relations would not be true if the number of bonds demanded by ordinary valence theory (3 in CN, 2 in CO, 2 in NO) were assumed;[16] matters would be still worse if in O_2 only 1 bond were assumed, in accordance with the spin theory of valence.

The facts just brought forward strongly suggest that instead of treating the electron pair as a unit bond, we should regard a *single bonding electron as the natural unit bond*, and an anti-bonding electron as a negative unit. Although it is customary to treat the electron pair as the normal unit bond, the idea that a special kind of bond known as the "one-electron bond" occurs in some molecules has also been in use. Pauling has recently discussed this (14), using especially H_2^+ as an example, and has also introduced the concept of a "three-electron bond" in certain molecules (in particular He_2^+, NO and O_2). But it seems probable that all such special concepts can better be reduced to terms of bonding and anti-bonding electrons. The one-electron bond is a

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[16] The existence of three valence bonds in CO has, however, often been assumed, first by ·Langmuir (J. Am. Chem. Soc. **41**, 1543 (1919)). Herzberg (3) first pointed out that it is given also by the present method.

single bonding electron, the electron-pair bond is two bonding electrons symmetrically related, while the three-electron bond consists of a pair of bonding electrons plus one anti-bonding electron.

In NO Pauling assumes one three-electron bond and two electron-pair bonds. The author believes it would be more nearly correct to speak of three electron-pair bonds and one negative one-electron bond, or in other words of six bonding electrons and one anti-bonding electron. More accurately still, there are eight bonding electrons and three anti-bonding electrons. The three-electron bond does not appear to be a natural *unit*. In O_2 Pauling assumes one electron-pair bond and two three-electron bonds. It seems to the author that it would be better to speak of three electron-pair bonds (one $\sigma 2p$ and two $\pi 2p$), or six bonding electrons, plus two anti-bonding electrons ($\pi^* 2p$).

In F_2, already discussed, there is one strong electron-pair bond, but there are also two rather weak electron-pair bonds and two equally weak pairs of anti-bonding electrons. Here the anti-bonding as well as the bonding electrons are symmetrically related in pairs.

That bonding electrons nearly always are found in symmetrically related pairs in chemical molecules can be explained on grounds of maximum stability (cf. Summary) without adopting the assumption that such pairing is a really fundamental characteristic of chemical bonding—still less that one member of each pair must be contributed by each atom, as London and Heitler's spin theory requires. An analysis indicating that the establishment of a symmetrical relation between two electrons is only incidental in the formation of an electron-pair bond, has already been given at the end of the section entitled "Application of Heitler and London method to H_2 and H_2^+. . . . ."

Arbitrariness of the concepts of valence and of bonding electrons

The molecules HeH, HgH, CaH and He_2^+ illustrate well the essential arbitrariness of the concept of valence and the impossibility of accepting it as corresponding to an always sharply de-

finable, whole number property of atoms. Likewise they indicate that, while it is usually convenient to think in terms of definite numbers of electrons of the three classes bonding, anti-bonding, and non-bonding, or of the two classes bonding and non-bonding, the concept of bonding power as a continuous variable is more fundamental. The discussion given earlier (p. 377) of the $\sigma 2s$, $\sigma^* 2s$, $\pi 2p$, and $\pi^* 2p$ electrons in F_2 also points to the same conclusion. An even more fundamental viewpoint is that indicated in the section entitled "Superfluity of the concept of valence bonds," on p. 369.

We may interpret the instability of HeH by saying that its two $1s$ electrons are non-bonding at moderate r values—since being so firmly bound by the He atom (ionization potential 25 volts), they have very little tendency to enlarge their orbits so as to go around the hydrogen nucleus—and that the $1s$ electron of the hydrogen atom is anti-bonding at moderate r values, because it must be promoted to $2p\sigma$ as $r \to 0$, a process which would require much energy.

The small but not inappreciable D of HgH may be explained as follows. The two $6s$ electrons of the mercury atom (ionization potential 10.4 volts) here show a rather small positive bonding power, since they are decidedly attracted by the H^+ nucleus. The hydrogen $1s$ electron acts as an anti-bonding electron, being promoted to a $6\,p\sigma$ orbit, which is considerably less firmly bound in HgH than the $1s$ orbit in H, as is shown by spectroscopic data. The net effect is a very weak bonding.

In CaH the relations are qualitatively similar to those in HgH, but the two $4s$ electrons of calcium (ionization potential 6.1 volts), being less firmly bound in the atom than are the $6s$ electrons in mercury, are more strongly attracted by the hydrogen nucleus, and may perhaps be called bonding electrons. At the same time the hydrogen electron, in this case promoted probably to $3d\sigma$, acts as an anti-bonding electron as before.

In He_2^+ the two $\sigma 1s$ electrons are shared by the two nuclei, which approach to 1.06×10^{-8}cm. when the molecule is in equilibrium, and act definitely as bonding electrons, while the $\sigma^* 1s$ electron, being vigorously promoted, has a strong anti-bonding action.

VI. SUMMARY

From the foregoing one can see that the facts described by the rules of valence, as well as some of the exceptions to these rules, can be understood very clearly in terms of the electron configurations[17] of the molecules and their atoms, at least for simple diatomic molecules and for some of the simpler polyatomic hydrides. The method is so far a semi-empirical one in that it very often makes use of spectroscopic and chemical data in determining the energies of binding of the various types of orbits.

London and Heitler's spin valence theory, when applicable, usually gives, somewhat fortuitously *in the author's* opinion, the same results as the present method. The latter gives, however, a detailed insight into what is going on in the formation of the molecule. It is also useful for excited states, while the spin theory fails. In those cases where the present method has to be guided by numerical calculations, as for $2H\ (1s) \rightarrow H_2(1s\sigma 2p\sigma, {}^3\Sigma)$, or by empirical data, as it does in concluding that two neon atoms repel each other, the same is true of Heitler and London's valence theory. In cases such as those of $O + O$, $F + F$, or $Ca + H$, where Heitler and London's theory is unsatisfactory, as well as in certain cases where ordinary valence theory fails (e.g., B_2H_6), the present method gives a clear understanding.

It is emphasized that the concept of a discrete, whole number property of atoms called valence, is less fundamental from the point of view of quantum theory than a continuous conception of chemical binding; likewise that the idea of a definite integral number of bonding electrons is not so fundamental as the idea that every outer electron has a certain bonding power (B.P.), positive or negative. More fundamentally still, one must recognize that it is not strictly possible to divide the energy of formation of the molecule into parts assignable to the separate electrons.

Nevertheless for practical purposes most electrons in most molecules can be definitely classified as bonding (large positive B.P.), *anti-bonding* (large negative B.P.), or *non-bonding* (very

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[17] For a detailed discussion of electron configurations in diatomic molecules, the reader is referred to an article (reference 4) in the January, 1932, number of Reviews of Modern Physics.

small B.P.). In agreement with Herzberg, it is concluded that the number of valence bonds according to ordinary valence theory is usually equal, at least in diatomic molecules, to half the difference between the numbers of bonding and of anti-bonding electrons. Probably in polyatomic molecules, however, anti-bonding electrons are less prominent than in diatomic molecules. Probably usually they are weaker in their action and approximately balanced by an equal number of weak bonding electrons, giving as net result a merely non-bonding effect.

It is argued that the primary unit in valence is a single bonding electron, with an anti-bonding electron as a negative unit, and with the symmetrically related electron pair as a very common secondary unit. The fact that valence electrons almost always occur in pairs in saturated molecules appears to have after all no fundamental connection with the existence of chemical binding. It can be adequately explained on the basis of the fact that, because of the Pauli principle and the properties of electron spin, each type of molecular orbit can be occupied by just two electrons. For example, if the orbit in question is of a bonding type, then naturally the stability of the molecule is greater when this orbit is occupied, if they are available, by two electrons than if it is occupied by only one while the second goes into an orbit of lower bonding power.

A clearer understanding of molecular structure, especially in hydrides, can often be obtained by dropping altogether the idea of atoms or ions held together by valence forces, and adopting the molecular point of view, which regards each molecule as a distinct individual built up of nuclei and electrons.

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EPINEPHRINE AND RELATED COMPOUNDS: INFLUENCE OF STRUCTURE ON PHYSIOLOGICAL ACTIVITY

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"The real object of chemistry is not to make gold, but to prepare medicines."—PARACELSUS (1).

I. INTRODUCTION

The relationship between the structure and behavior of physiologically active compounds is still poorly understood. That there is such a relationship is well evidenced by the information available concerning individual series or homologs, as, e.g., the alcohols, amines, phenols, purines, barbituric acid derivatives, acridine dyes, chaulmoogric acid and its analogs and the piperazines, but the extent to which these various and varying groups may be interrelated still remains to be determined. Perhaps some day there will come another Mendeléeff, a man with a genius to appreciate existing knowledge, combined with a capacity to organize it into a second "periodic table," namely, a classification that will coördinate the physiological significance of the functional groups and at the same time preserve a regularity in structural sequence. The far-reaching consequences that will follow the formulation of such a "law" cannot be imagined, for it will serve as the course of illumination that will extend many times the horizon in a field of endeavor now shrouded in semidarkness. However, pending the attainment of such a coveted goal, the modern iatro-chemist must of necessity content himself with the limited view now possible.

One of the earliest, and possibly the best known, studies in

correlating chemical constitution and pharmacological behavior was made with compounds that produce a rise in blood pressure. The extent and nature of such studies immediately suggest themselves at the mere mention of such names as Abel, Barger and Dale, and Chen, or of compounds like tyramine, epinephrine and ephedrine.

Interest in these substances was aroused when Oliver and Schaefer in 1894 (2) and Scymonowicz independently in 1895 (3) found that extracts of the suprarenal glands, which are located above the kidneys and the importance of which was first observed by Addison in 1849, produced a rise in blood pressure when injected into the blood vessels of animals. This discovery aroused the interest of chemists, physiologists, and pharmacologists the world over and was the beginning of an intensive study of the gland and an effort to isolate its highly active principle. Progress was quickly made and a keen rivalry developed among the various workers. Among the pioneering investigators were Abel of this country and v. Fürth in Germany. The former was the most active and it was from his laboratory that most of the early information about the chemical nature of the active constituent of the glands came.

Abel and Crawford (4) separated the active hormone from the tissues in the form of a polybenzoyl derivative; this, when decomposed in an autoclave by means of hot dilute sulfuric acid, resulted in the formation of an active sulfate possessing all the characteristic activities of the glandular extracts to a very high degree (5, 6). From the analysis of the sulfate and other derivatives Abel concluded that the active principle was represented empirically by the formula $C_{17}H_{15}NO_4$ (7).

During the fall of 1900, while Abel was still at work on his processes, his laboratory was visited by Takamine, who became very much interested in the work and upon inquiry was informed that the process of isolation "could no doubt be improved and simplified." Takamine returned to his own laboratory, prepared concentrated extracts of the glands, and by the addition of ammonia (the base employed by Abel in precipitating his epinephrine) obtained burr-like clusters of crystals. These crystals were not,

however, identical with those isolated by Abel. Takamine called his product adrenaline and gave it the formula $C_{10}H_{15}NO_3$ (8, 10). The product was crystalline epinephrine, but still not yet pure. It remained for Aldrich (11, 12) to show its true formula, which is $C_9H_{13}NO_3$. The error in Abel's product was, as Aldrich pointed out and Abel (13) had already demonstrated, that it contained a benzoyl radical, for "it is interesting to note . . . . that if we subtract a benzoyl residue from Abel's formula for epinephrine, $C_{17}H_{15}NO_4$, we obtain formula $C_{10}H_{10}NO_3$, which is not far removed from that of adrenalin" (14), and which agrees very well with the formula $C_{10}H_{15}NO_3$ proposed by Takamine. Abel suspected that this benzoyl group, which could not be hydrolyzed off, was attached to the amino nitrogen[1]—"an unusual circumstance in any event" (15).

The difficulties which confronted Abel are quite understandable. A trail blazer in science, attempting to isolate the active principle of an unusually active glandular extract, found that by a "benzoylation" process he could obtain, in a highly purified form, a substance that retained all the characteristic physiological activity of the extract itself, and to add to the difficulty the activity compared favorable with that of the pure principle; this substance actually was a monobenzoyl derivative of the product sought. What other secretion could undergo so drastic a modification as this without having its characteristic action destroyed or at least greatly changed? Such was the combination of circumstances which conspired against a pioneer. However, the scientific world today recognizes the value of Abel's contributions and he is now generally accorded the credit due him, namely, that of having isolated the first hormone.

[1] In the light of all that is now known it may not be out of place to suggest that this monobenzoyl derivative of epinephrine, which was obtained by Abel and his co-workers, is not the N-benzoyl derivative but rather that the benzoyl group entered the catechol nucleus, for acylation of the amino portion of compounds of this type tends to destroy their pressor activity and it is difficult to conceive, as Abel points out, how an N-benzoyl derivative could resist hydrolysis under the conditions employed. On the other hand, the entrance of the benzoyl group into the aromatic nucleus through an adaptation of the Fries rearrangement, would account for its nonremoval and probably would not interfere so greatly with its physiological activity. Such a reaction is quite common with phenols.

The principle has been given various names—epinephrine by Abel, suprarenine by v. Fürth (16), who isolated it through a ferric chloride complex, and adrenaline by Takamine. The term epinephrine has been adopted by the U. S. Pharmacopoeia. Rogoff (17) suggests that "it is preferable to employ the term 'epinephrine' as indicating the physiological secretion from the adrenal medulla, and to use 'adrenalin' when referring to the commercial product."

Once epinephrine was isolated, its formula was quickly established. Even before the existence of the hormone was suspected, a color reaction of the glandular extract led Krukenberg (18) to remark about its similarity to catechol, and Moore (19) believed that this color-producing body and the blood pressure raising principle were identical. Takamine, by fusing his adrenaline with alkali, obtained catechol and catechuic acid. v. Fürth (16, 20) added the observation that the molecule contained a methylamino group and proposed that it contained a catechol nucleus. Pauly, on the basis of his discovery that epinephrine contains an asymmetric carbon atom (21) and of other contributing considerations, suggested that the diphenolic nucleus was attached to one of the five theoretically possible side chains,

$$—CH_2—CH\begin{smallmatrix}OH\\ \\NHCH_3\end{smallmatrix} \qquad —\underset{\underset{OH}{|}}{\overset{\overset{CH_3}{|}}{C}}—NHCH_3 \qquad —\underset{\underset{\underset{CH_3}{|}}{O}}{\overset{}{CH}}—NHCH_3$$

I   II   III

$$—\underset{\underset{OH}{|}}{CH}—CH_2NHCH_3 \qquad —\underset{\underset{NHCH_3}{|}}{CH}—CH_2OH$$

IV   V

but considered groupings IV and V as most probable, since they would account for the formation of skatole or pyrrole derivatives which previous investigators had observed among the decomposition products. Jowett (22), with potassium permanganate, ob-

tained oxalic and formic acids and methylamine; after methylation and subsequent oxidation he obtained veratric acid and trimethylamine, proving thereby the presence of the complexes $C_6H_3(OH)_2C\equiv$ and $-NHCH_3$ in the original base. From these results he proposed the three possible formulas

| VI | VII | VIII |

and argued that formula VI was the most probable since a substance with formula VII should, after methylation and subsequent oxidation, give homoveratric acid, $(CH_3O)_2C_6H_3CH_2COOH$, and formula VIII does not so readily explain the formation of pyrrole derivatives.

Stolz, convinced by the evidence of his predecessors that either VI or VIII of Jowett's formulas was correct, set out to synthesize VI.   He prepared chloroacetylcatechol, $(HO)_2C_6H_3COCH_2Cl$ and condensed it with methylamine, thus obtaining methylaminoacetocatechol (IX).

IX

When he found that this ketone possessed qualitatively all the physiological activity of the hormone itself and that the reduction of the aminoketone gave a product that produced an even greater characteristic epinephrine activity, there could be no longer any doubt about the chemical identity of epinephrine (23, 24).   The syntheses of related substances by Dakin (25) and by Friedmann (26) served to confirm these conclusions.

Hence, it is seen that epinephrine is a comparatively simple substance, being the levo form of (3,4-dihydroxyphenyl)—1-methylamino-2-ethanol (X).

OH

OH

H—C—CH₂NHCH₃

OH

Epinephrine

X

Probably no other compound has been so extensively used in physiological and chemical investigations as has this first-known hormone. A bibliography of epinephrine would form a large volume, as reference to the indices of any abstract journal will show. Some idea of the importance of this compound may be obtained from the graphic tabulation of the number of references in the indices of the annual volumes of Chemisches Zentralblatt (figure 1). From even a cursory survey of these numerous references one may readily see how important a substance it has become in therapeutics, diagnoses, and physiological experimentation and even as a chemical reagent.

Epinephrine is an extremely active substance, and has been found biologically to exert an effect on the isolated frog heart in dilutions as low as one part in five billion (27). The isolated frog heart, rendered hypodynamic by aconitine, was susceptible at even greater dilutions—one part per trillion (28, 29). The epinephrine content in the suprarenal glands averages, for healthy persons of middle age, about 4 mg. for each gram of dried gland (30). In the fresh glands of animals it varies from 150 mg. per thousand glands of the guinea pig to 2440 mg. per thousand glands of the horse (31). Barger has calculated that the glands of twenty million beeves are necessary for the isolation of a ton of the active principle (32). The average normal output of epinephrine from the glands as determined from 103 cats was 0.000226 ± 0.0000007 mg. per kilogram of body weight per minute; in 32 dogs the

figure was found to be 0.000227 ± 0.00000096 mg. per kilogram of body weight per minute (33).   Since such small amounts of epinephrine are determined or estimated only with great difficulty, too much reliance cannot be placed on these figures.

The exact function of epinephrine does not appear to be completely or definitely determined, owing in part to the difficulty of removing all chromaffin tissue from experimental animals.
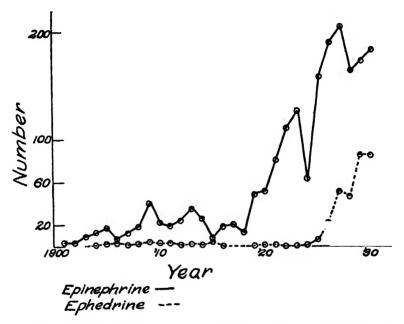
FIG. 1. REFERENCES TO EPINEPHRINE AND EPHEDRINE FOUND IN THE INDICES OF CHEMISCHES ZENTRALBLATT

Its therapeutic value is based in part on the effects its produces, viz.: its action on smooth muscle fibres, which affords relief in asthmatic spasms, hay fever, severe colds, and causes it to act as a constrictor on the arteriole muscles; its hyperglycemic effect, which causes the conversion of muscle and liver glycogen into glucose; and mydriasis, i.e., dilatation of the pupils.   Recently the oxidation products of epinephrine have been shown to exert a peculiar catalytic effect on proteins (34, 35).

Sollman (36) states that "the striking actions of epinephrine invite the theory that it plays an important rôle as a hormone for maintaining the tone of the sympathetic system, particularly of the blood pressure; and that it is conceived in pathologic conditions of the sympathetic system." Guyer (37) has summed up the functions of epinephrine in the following words: "In the body it is of great importance in maintaining muscular tone; the proper amount keeps the blood vessels suitably contracted and blood pressure normal..... Insufficiency of adrenaline .... results in lowered blood pressure, lack of muscular tone and the general loss of strength and 'nerve' which is characteristic of neurasthenia, 'shell-shock' and related ills.   In general, adrenaline affects the same structures of the body that the sympathetic nervous system does; namely, the heart, blood vessels, kidneys and other viscera and the involuntary muscles. . . . . Injection of adrenaline into the blood tends to increase the quantity of sugar in the blood through release of the sugar from liver glycogen.   It apparently counterbalances the action of insulin."   Cannon (38, 39) has advanced a theory that any of the major emotions, such as pain, fear and anger, result in a large outpouring of epinephrine from the adrenal glands into the blood stream in order to mobilize the defense mechanisms of the individual.   This theory has been extended in a most fascinating manner by Berman (40), and rather remarkable properties are ascribed to the "glands of combat and fight;" for instance, not only is courage determined by epinephrine secretion, but also neurasthenia, "the great American disease," may be directly traced to epinephrine insufficiency. Interesting and plausible as Berman's hypotheses may be, one must not forget that his book, which "adds somewhat to the gaiety but little to the sanity of the time," contains "speculations, unfounded or extremely doubtful generalizations ·. . . . so skillfully interwoven with the facts" that it must not be taken seriously (41).   As for Cannon's original defense mechanism hypothesis, Rogoff (17) considers it based on very meagre and indirect evidence.

Since epinephrine is a phenol, it might, *a priori*, be expected to possess bacteriostatic or even bacteriocidal powers.   Its "phenol

coefficient" seems not to have been determined and its germicidal value probably is not very large, for the natural product, obtained from tissues, must be sterilized before it can be used clinically (42). Sterilization is also necessary for its preservation. Nevertheless epinephrine has been found to have an antiseptic effect on the organisms in "brûlures" (43), and it appears to have some merit in neutralizing the effect of tetanus and diphtheria toxins (44, 45, 46, 47, 48, 49, 50, 51, 52). Derisi (52a) reports favorable results from oral administration of epinephrine in the treatment of infectious diseases such as grippe, typhoid, paratyphoid, diphtheria, scarlet fever and measles; however, he explains these favorable results on the supposition that these diseases have caused adrenal deficiencies.

Epinephrine is administered usually by injection subcutaneously, and rarely intravenously. Administration by way of the rectum produces no, or only very slight, effects (53). Given orally it has, as a rule, no influence on the blood pressure (54, 55, 56, 57), although some observers have recorded such effects. Such exceptions may, for the present, be regarded as unusual, for if the oral administration of epinephrine produced a characteristic effect on the blood pressure that fact should, from the hundreds of observations, be more obvious than is now the case. This oral inactivity is generally attributed to the inability of the easily modified epinephrine molecule to withstand the rather strenuous processes of absorption from the intestinal tract. This view, however, appears unwarranted, for while epinephrine may, *per os*, have no general effect on the blood pressure it does produce other systemic results. It will cause a rise in blood sugar (55, 60, 61, 62, 63) and in general will increase the basal metabolic rate (64), thus indicating that the theory of the rapid and complete destruction of the epinephrine in the alimentary tract will not explain the lack of action on the blood pressure, for it is sufficiently capable of producing some of the other characteristic epinephrine responses. Other considerations, which will be discussed later, show that this inactivity is attributable rather to a deficiency in minimum structural essentials that are necessary for oral activity.

While epinephrine is a very important secretion of the adrenal

glands its real function in vital economy is as yet incompletely known. Addison's disease, which is always fatal, does not seem to be associated with a lack of epinephrine (65). In fact there is some question whether the hormone is really necessary in sustaining life (36, 66). Stewart, Rogoff and their co-workers report that the secretion of epinephrine is not indispensable to life and that they were able to suppress its secretion completely without causing any detectable harmful influence (67, 68, 69). However, the *complete* suppression of the functioning of the epinephrine-forming tissues has probably never been successfully accomplished (70), because epinephrine even in the smallest quantities is so very active (6). Whatever may ultimately be learned about the function of epinephrine in the economy of living tissues, it would appear that a substance so active must, from the very fact of its presence, play at least a very important if not an indispensable rôle.

Nor is epinephrine the only hormone of the glands. Extracts of the suprarenal cortex have been shown to be necessary to life. Popular interest was aroused in these extracts when Coffey and Humber (71, 71a, 71b) reported that they were able to cure carcinoma with them; Itama and McDonald (72), however, were unable to duplicate this work. Various extracts of the suprarenal cortex have, however, been used with much promise in the treatment of Addison's disease and in maintaining the normal health of adrenalectomized animals (73, 74, 75, 76, 77, 77a).

While the interest in epinephrine is usually associated with its presence and its function in the higher animals, it is found in structures other than the suprarenal glands. Abel and Macht (78) found that the venomous secretion of the Brazilian toad, the *Bufo agua*, used by the natives as an arrow poison, contained nearly 7 per cent epinephrine. While the death-dealing principle of the venom is a glucoside, *bufagin*, one can but wonder at the reason for the presence of the hormone, particularly in such high concentration. In 1916 Schimizu (79) reported the presence of an epinephrine-like body in "Senso" (Japanese) or "Ch'an Su" (Chinese), the dried venom of the Chinese toad. Jensen and Chen (80, 81) identified this substance as epinephrine and ob-

tained 350 mg. from 150 g. of Ch'an Su. It is not unlikely that the venom of all toads contains epinephrine, for Epstein and Gunn (79a) found an epinephrine-like substance also in the parotid secretion of the African toad, *Bufo regularis*. Collip (70) has demonstrated the presence of an epinephrine-like substance in the prostate gland of the bull.

Various methods for the synthetic preparation of epinephrine have been developed, most of them involving the reduction of adrenalone, the corresponding aminoketone. This desired intermediate ketone may be prepared by allowing halogenoacetylcatechol

$$\text{OH} \atop \text{OH} \atop \text{COCH}_2\text{X}$$

to react with methylamine (23, 82); reduction of the resulting methylaminoketone, by appropriate methods, gives epinephrine (83, 84, 85). Several have tried condensing the methylamine directly with the halogenocarbinol (86, 87, 88),

$$\text{OH} \atop \text{OH} \atop \text{CHOHCH}_2\text{X}$$

but apparently were less successful in obtaining the desired product. An entirely new and more recent synthesis was made by Nagai (89), who reduced the condensation product from nitromethane and the diacetate of protocatechualdehyde with zinc and acetic acid in the presence of formaldehyde and obtained as the end product the desired epinephrine.

Much of the epinephrine used clinically is obtained from synthetic sources. The synthetic racemic substance is resolved into its levo and dextro components; the levo form is as effective as that obtained from natural sources and is accepted by the Pharmacopoeia. The dextro portion is racemized, by proper heating in a dilute acid solution, and the levo isomer again removed. In

this manner it is possible eventually to convert substantially all the synthetic product into acceptable *l*-epinephrine. However, the chief source of supply of epinephrine remains the suprarenal glands of beeves.

While the fame of epinephrine was steadily increasing, it was with considerable interest that the report of Abelous, Ribaut, Soulie and Toujan (90) in 1906 was received. They observed that extracts of putrified meat also contained a substance that produced a rise in blood pressure. Barger and Dale (91) identified the active ingredients as two definite compounds, isoamylamine and tyramine. Since both may be derived, by putrefactive processes, from the protein acids, leucine and tyrosine, respectively, these scientists were led to investigate first other bases of putrefactive origin and finally all substances structurally related to tyramine and epinephrine. Their results are reported in that classic which first showed what an intimate connection there is between the physiological activity of compounds possessing structural similarity (92).

Because all of the substances investigated caused a rise in the arterial blood pressure by constricting the muscular lining of the arterioles, Barger and Dale described them as "sympathomimetic," i.e., minicking sympathetic stimulants, a term which is now in the vocabulary of all physiologists and pharmacologists.

During the fourteen years following this work of Barger and Dale nothing of particular interest developed; known compounds were more intensively investigated and occasionally new ones without especial merit were introduced. Meanwhile epinephrine was becoming more firmly established.

In the autumn of 1923, at Peking Union Medical College, a decoction made from Ma Huang, a plant of the ephedra species, was injected into the vein of an anesthetized dog remaining alive at the end of the laboratory exercise (93). The curious student who made the experiment was Chen, and the results of that casual injection have been world-wide in their effect. Chen was quick to see that this substance, which had been used by the Chinese for over 5000 years as a sedative, diaphoretic and circulatory stimulant, produced an action on the blood pressure simulating that

of epinephrine. Further investigation of the active principle revealed its virtues and finally led to its introduction into modern medicine, where it has assumed an increasingly important rôle. The active principle of this old Chinese drug plant was isolated in 1885 by Nagai who gave it its name, ephedrine.[2]

Previous to 1923 the alkaloid had been investigated physiologically, but apparently in such doses that the toxic effects predominated. It had been found satisfactory as a mydriatic but never attained clinical prominence as such. It remained for Chen and his collaborators to discover its virtues. The extent to which Chen's observations have revived interest in this little-known alkaloid may be seen from figure 1 which gives a graphic summary of the number of references to ephedrine in the annual indices of Chemisches Zentralblatt. Since Chen and Schmidt (93), the modern sponsors of ephedrine, have recently written a monograph covering the history, chemistry, and drug action of this old Chinese alkaloid, it would be mere repetition to discuss it further here.

II. PHARMACOLOGICAL METHODS

It is beyond the province of this paper to discuss the methods of animal experimentation. These may be found described in any appropriate standard reference. But it should be emphasized that data obtained by biological means are not quantitative in the sense in which the analytical chemist understands the term (94), and that in evaluating the relative potencies of a series of physiologically active compounds many factors must be considered, of which the following deserve especial mention: (a) variation in individual animals; (b) variation in characteristic responses in different species of animals; (c) influence of experimental conditions and procedure; (d) significant differences in biochemical mechanisms through which individual reagents produce their effects.

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[2] In the United States the pronunciation commonly heard is e-phĕ'-drine or, sometimes e-phē'-drine, with the accent on the second syllable. The name of the species from which the alkaloid is obtained is ĕph'-e-dra, and the American dictionaries give its active principle the pronunciation ĕph'-e-drine. See also Nielson: Am. Druggist, Jan. 28, 1928, p. 90.

Even within a single species of animals there are very wide variations, and in some instances the animal may react in a manner that is qualitatively different. The results obtained from one animal may be indicative of a trend but they should never be accepted as the sole basis for a positive conclusion. The greater the number of animals used, the more reliable is the summation of findings.

Animals of different species do not necessarily give the same response. For example, a cold-blooded animal may react quite differently from a warm-blooded one; or a rabbit may be much less sensitive to a given compound than, say, a dog. The larger the variety of animals on which a drug has been tested the more certain one can be as to what may be expected of it. That this factor of species variation must always be considered is strikingly illustrated by Chen and Poth's observation that the mydriatic action of ephedrine is much more pronounced in Caucasians than in Chinese or Negroes (95), and by the fact that ephedrine mydriasis is much more effective in individuals with light irises than in those with dark irises (96).

Variations in experimental technique must always be considered, such as the type, size, age, sex, previous history, and the nature and depth of anesthesia of the experimental animal; the rate and method of administration of the chemical, namely, whether given orally, rectally, subcutaneously or intramuscularly; and the size of the dose. All these produce their own peculiar effects on the qualitative and quantitative nature of the physiological response. The adoption of a "standard" method or procedure for pharmacologists and physiologists would make all comparative results more reliable, but it might also keep hidden any peculiar drug action that another technique would reveal. For the purposes of comparison the different results of a single experimenter or author are the most reliable.

The biological mechanism through which a substance produces its effect cannot be ignored. Thus, epinephrine is distinctly "sympathicotropic," that is, it produces its effects by stimulating the sympathetic nervous system (97, 97a). On the other hand, while ephedrine may also stimulate the sympathetic system

according to some (98, 99), it is quite definitely "musculotropic," producing its effects by stimulating the muscles. Here, then, are two substances both of which affect the circulatory system but through quite different channels. Any real comparison must also consider such differences.

Keeping in mind, throughout the following pages, factors such as these, one can see that many apparent inconsistencies and contradictions are unavoidable in tracing the thread of relationship that weaves through a series of compounds having structural elements in common.

III. ALIPHATIC AMINES

The physiological effect of aliphatic amines may be exhibited in various ways (100, 101). Ammonia in small doses is a respiratory stimulant, but larger doses cause convulsions; it produces a rapid but very transitory depression of the blood pressure (102). As alkyl groups replace the hydrogen atoms of ammonia the stimulating action is diminished, becoming less as the size of the alkyl group increases; as the alkyl chain becomes longer, a depressant action on the heart and convulsions of spinal origin appear, the depressant action being perceptible even in isoamylamine.

The lower members of the series of aliphatic amines possess practically no pressor properties; in fact, they appear to be depressors rather than pressors (102, 103, 104, 105). While Barger and Dale did observe that large doses produced perceptible rises, they found that the record was complicated by volume effects (92). Abelous and Bardier credit trimethylamine with a "urohypertensive" action 1/200th as great as that of isoamylamine (106), an effect which Barger and Dale dismiss as negligible. Jackson, however, has obtained an excellent tracing which shows trimethylamine to be active as a pressor and also as capable of producing constriction of the bronchioles. A dose of 0.25 ml. (concentration not stated) given to a decerebrate dog produced a maximum rise in blood pressure of 63 mm. of mercury (107). Very recently Mercier (108) observed that the blood pressure of a chloralosed dog receiving 20 mg. per kilogram of trimethylamine (as hydrochloride) in the saphenous vein fell about 40 mm. of

mercury, and after about twenty seconds rose to 64 mm. above normal for a minute or longer.

Barger and Dale (92) examined the following twenty-one aliphatic amines: methylamine, ethylamine, propylamine, isopropylamine, isobutylamine, n-butylamine, isoamylamine, n-amylamine, n-hexylamine, n-heptylamine, n-octylamine, n-nonylamine, undecylamine, tridecylamine, cyclohexylamine, diethylamine, isoamylmethylamine, diisoamylamine, trimethylamine, tetraethylammonium iodide, pentamethylene diamine (cadaverine). They found that pressor activity began with n-butylamine; n-amylamine was much more active, while maximum activity of the whole series was observed in n-hexylamine, for n-heptylamine was slightly but distinctly less active, and octylamine even less active; pressor activity was still apparent in the higher homologs, even in tridecylamine; however, with the higher members increasingly greater toxic disturbances interfered with the purely sympathomimetic results. The normal chains were found more potent than the branched or isochains; thus isoamylamine, while several times more active than n-butylamine, was weaker than n-amylamine, and the effect of isobutylamine was doubtful.

Hanzlik (105) found that in atropinized dogs the butylamines caused a fall in blood pressure, and Trendelenburg (103) reported that isoamylamine did not always cause a rise in the blood pressure of rabbits.

Repeated doses of these amines produced, according to Barger and Dale, rapidly diminishing effects, that is, a second and equal dose did not produce the same response as was obtained from the first, and the response from the third dose was still less.

Introduction of an additional amino group into n-amylamine, giving cadaverine, converted it into a depressor, thus completely reversing the physiological effect. If the hexylamine was converted into a cyclic derivative, e.g., into cyclohexylamine, the physiological response was slower in appearing and was more prolonged, but otherwise resembled that obtained with the open chain compound (92).

The primary amines were more active, both as pressors and in their effect on the cat uterus, than were the secondary and

TABLE 1

Aliphatic amines

BASE	TOXICITY—M.L.D.*	PHYSIOLOGICAL ACTION
Methylamine	200–300 mg. per 100 g. of frog, subcutaneous (103) 300–400 mg. intravenous to rabbit not fatal (103) 2000 mg. subcutaneous to rabbit not fatal (103) 200–300 mg. of hydrochloride or sulfate subcutaneous to guinea pigs or rats (103)	Injection into mammals causes rapidly disappearing depression in blood pressure (103) 0.005–0.010 g./kg. intravenous to rabbits caused depression of 14 to 16 mm. Hg in blood pressure (110) Less depressant than ammonia (102)
Dimethylamine	0.6 g. of base (given as salt) fatal to rabbits (103) 4 g. orally to rabbits fatal (104)	Gives transitory depression in blood pressure (103) Less depressant than methylamine (102)
Trimethylamine	0.1–0.2 g. to frogs (103) 1 g./kg. to rabbits kills in 4 hrs. (103) 6 g. of base subcutaneous to rabbits (103) 0.15–0.20 g./kg. to frogs (104) 0.4 g./kg. intravenous to rabbits (104) 0.8 g./kg. subcutaneous to rabbits (104)	Action resembles methylamine and dimethylamine in rabbits 0.2 g./kg. intravenous gives fall in blood pressure for several minutes, then gives enormous rise (111) Less depressant than dimethylamine (102) 1/200th as great a pressor as isoamylamine (106) Good pressor (107) The hydrochloride injected intravenously gives preliminary fall followed by rise in blood pressure (108)
Ethylamine	0.35 g./kg. of hydrochloride intravenous to rabbits, no toxic symptoms (103) 2 g. of hydrochloride fatal to rabbit (103) 0.5 g. of hydrochloride fatal to rat (103)	Differs only quantitatively from methylamine (103)

* Minimum lethal dose.

TABLE 1—*Concluded*

BASE	TOXICITY—M.L.D.*	PHYSIOLOGICAL ACTION
Diethylamine	Less toxic than dimethyla-mine (103)	Inactive (103)
Propylamine		Inactive (103)
Isopropylamine		Inactive (103)
Dipropylamine	Ten times as toxic as di-ethylamine (103)	
n-Butylamine	0.60 ml. of 1 per cent solu-tion subcutaneous to white rats (105)	In atropinized dogs cause fall in blood pressure
Di-*n*-Butylamine	0.47 ml. of 1 per cent solu-tion subcutaneous to white rats (105)	Cause increase in cardiac volume and decrease in kidney volume
Tri-*n*-Butylamine	0.45 ml. of 1 per cent solu-tion subcutaneous to white rats (105)	Smooth muscle stimulant (105)
Isoamylamine	150–200 mg. of hydrochlo-ride per 100 g. of frog 250 mg./kg. not toxic to rabbits 1.5 g. of sulfate killed rat 1.8 g. of hydrochloride killed rabbit (103)	Does not always give blood pressure rise in rabbits (103)
Amylamine		More active pressor than isoamylamine (92)
n-Hexylamine		Most active of aliphatic amines (92)

tertiary amines (104). Methylisoamylamine was about half as active as isoamylamine (92).

Many of the aliphatic compounds, particularly those with a normal chain, may be found among various protein decomposition products (109).

In table 1 is given a summary of the information relative to the aliphatic amines.

The aliphatic aminoalcohols, that is, the amines with an alcoholic function added, are compounds of biochemical interest. Ethanolamine, $CH_2OH \cdot CH_2NH_2$, and ethanol-trimethylammonium hydroxide or choline, $CH_2OHCH_2N(CH_3)_3OH$, form constituent portions of the lecithins. Higher homologs have recently been prepared by Kanao (112). These, when converted into higher secondary bases, exhibit marked anesthetic properties. Thus, all of the following were found to be anesthetic.

$$(CH_3)_2CHCH_2CHCHOHCH_3$$
$$|$$
$$NHCH_2C_6H_5$$

$$(CH_3)_2CHCH_2CHOHCH(CH_3)NHCH_2C_6H_5$$

$$CH_2OHCH_2NHCH_2CH_2CH(CH_3)_2$$

$$(CH_3)_2CHCH_2NCH_2CH_2OH$$
$$|$$
$$C_6H_5$$

$$CH_2OHCH_2N(C_6H_5)_2$$

$$CH_3CHOHCH_2N(CH_2CH_2CH(CH_3)_2)_2$$

$$CH_3CHOHCH_2N(CH_2C_6H_5)_2$$

$$CH_3CHOHCHCHOHCH_3$$
$$|$$
$$NHCH_2CH_2CH(CH_3)_2$$
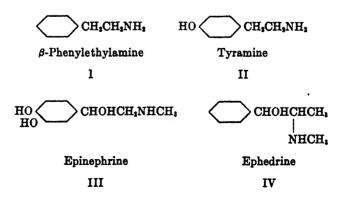
Other aliphatic aminoalcohols are of interest because their benzoic or substituted benzoic acid esters form anesthetics of the novocaine and stovaine type.

$$CH_2CH_2N(C_2H_5)_2$$
$$|$$
$$O$$
$$|$$
$$CO$$

$$\overset{C_2H_5}{\underset{|}{CH_3-C-CH_2N(CH_3)_2}}$$
$$|$$
$$O$$
$$|$$
$$CO$$

Novocaine   Stovaine

However, since ethanolamine does not have any marked effect on the blood pressure—higher homologs do not appear to have been investigated (25)—an extended discussion will not be given here.

IV. AROMATIC DERIVATIVES

While some of the fatty amines are capable of producing a hypertensive effect, the commoner and more effective compounds are aromatic derivatives. The presence of the aromatic nucleus is significant. Dakin (25), in his synthesis of epinephrine, found that catechol has the capacity to cause a rise in blood pressure, whereas ethanolmethylamine, $CH_2OHCH_2NHCH_3$, the side chain portion of epinephrine, has not. Tainter (113) also found catechol to have definite hypertensive properties. Even chloroacetyl catechol

$$HO\text{—}\bigcirc\text{—}COCH_2Cl$$
$$HO$$

is slightly active. Other dihydroxybenzenes, resorcinol and quinol, show no such effect; or, if one of the phenolic functions of catechol is covered, e.g., by acetylation, no action is obtained (114). Except for catechol the aromatic nucleus in itself is not sufficient to produce the desired physiological effect, but its presence is most important.

$$\bigcirc\text{—}CH_2CH_2NH_2 \qquad HO\text{—}\bigcirc\text{—}CH_2CH_2NH_2$$

β-Phenylethylamine   Tyramine

I   II

$$HO\atop HO\text{—}\bigcirc\text{—}CHOHCH_2NHCH_3 \qquad \bigcirc\text{—}CHOHCHCH_3\atop NHCH_3$$

Epinephrine   Ephedrine

III   IV

A comparison of the structure of the better known, natural pressors reveals the following facts:

The skeleton of β-phenylethylamine (I) is common to all.

I and II are primary bases, while III and IV are secondary bases.

III and IV have a secondary alcoholic hydroxyl group, which is lacking in I and II.

I, II and III are ethane derivatives, while IV is a derivative of propane.

II and III are phenolic; II is a mono-para-phenolic and III is a di-meta-para-phenolic compound.

What is the significance of these differences? What are the differences in the physiological behavior of the substances? What is the change in the pharmacological action when still other modifications are introduced? In the following pages the effect of such structural modifications on the physiological activity will be considered.

V. VARIATION FROM THE β-PHENYLETHYLAMINE SKELETON

Why does Nature, in making her products, attach the aromatic nucleus to one carbon atom and the amino, or substituted amino, group to an adjacent carbon atom? What would be the effect if this characteristic arrangement should be disturbed? Barger and Dale (92) were the first to investigate this problem; they compared a series of compounds in which the relative positions of the phenyl and the amino portions varied. They found aniline to be without effect, benzylamine to be slightly active, α-phenylethylamine to be slightly more active and β-phenylethylamine to have maximum activity, while γ-phenylpropylamine was again much less active. From these results the authors concluded that the optimum constitution for sympathomimetic activity is that in which the phenyl group is attached to one carbon atom and the amino group to an adjacent carbon atom. Although these authors made no allowance for the successive introduction of an additional methylene group into the side chain, subsequent work has proved them substantially correct in concluding that "the optimum constitution of a fatty-aromatic amine for the production of sympathomimetic action is, therefore, that which is found in adrenaline itself, viz., a benzene ring with a side chain of two carbon atoms, of which the second bears the amino group."

From his examination of hordenine homologs

$$HO\langle\ \rangle CH_2CH_2N(CH_3)_2$$

Hordenine

Heinz reported to v. Braun (115) that as the number of carbon atoms separating the amino group from the aromatic portion of the molecule was increased from two to three the pressor effect was completely reversed, i.e., the substance became a depressor; and as the length of the side chain was increased to four or five

TABLE 2

Phenylpropylamines (119)

AMINE	TOXICITY OF HYDROCHLORIDE		PRESSOR ACTIVITY OF HYDROCHLORIDE
	Rats—subcutaneous	Rabbits—intravenous	Dogs—intravenous
	mg./kg.	*mg./kg.*	
$C_6H_5CH_2CH_2NH_2$	450	60	1 mg./kg.—good rise which persisted for 20 minutes
$C_6H_5CH(NH_2)CH_2CH_3$...........	1000	50	1 mg./kg.—very slight rise
$C_6H_5CH_2CH(NH_2)CH_3$...........	25	25	1 mg./kg.—rise equal to that of $C_6H_5CH_2CH_2NH_2$; effect persisted longer
$C_6H_5CH_2CH_2CH_2NH_2$............	100	50	1 mg./kg.—medium transitory rise
$C_6H_5CH(CH_3)CH_2NH_2$...........	500	50	Good pressor; also active on oral administration

carbon atoms the depressing effect became correspondingly greater. Pick (116, 117), working with the same series of compounds, did not observe this reversal, but he did find a weakening in the pressor effect. Hasama (118), comparing the effects produced by the two isomeric phenylethanolamines, $C_6H_5CH(NH_2)$-CH_2OH and $C_6H_5CHOHCH_2NH_2$, found the former inactive as a pressor, whereas the latter is a very potent pressor. Tainter (118a) observed that if the chain separating the amino group from the aromatic nucleus in 3,4-dihydroxyphenylethylamine, $(HO)_2C_6H_3CH_2CH_2NH_2$, was increased to three carbon atoms the pressor activity was diminished by two-thirds. Hartung and

Munch (119), wishing to obviate the criticism of the series studied by Barger and Dale, studied the pressor effects of four isomeric phenylpropylamines. The results, summarized in table 2, show convincingly that the optimum pressor activity is obtained when the phenyl and amino groups are attached to each of two adjacent carbon atoms.

It is of interest to note in this connection that if in histamine, which lowers the blood pressure, the length of the side chain is decreased to one carbon atom, or increased to four carbon atoms,

Histamine

the effect on the blood pressure is distinctly lessened (120). Thus it becomes evident that in the relative positions of the aromatic portion and the amino group of compounds of this type, nature cannot be improved upon.

VI. MODIFICATIONS IN THE AMINO GROUP

Among the natural compounds belonging to the type under consideration will be found primary, secondary and tertiary bases. Tyramine is a primary base, while hordenine is the corresponding tertiary base; epinephrine is a secondary base, as is also ephedrine. Associated with ephedrine have been found in small amounts the corresponding primary and tertiary bases (121, 122, 123). Hence, the question naturally arises, what is the difference physiologically between the respective types of bases?

Barger and Dale (92) investigated this phase also in their classical studies. They reported that when β-phenylethylamine, tyramine and phenylethanolamine were converted into the corresponding secondary methylated bases there was no appreciable change in pressor potency. Perhaps the comparative determinations lacked the refinement necessary to reveal any differences that may exist, for subsequent observations by Chen, Wu and Henriksen (124) showed that methylation of phenylethanolamine,

that is, conversion into a secondary amine, decreases its activity very markedly.

In the case of 3,4-dihydroxyphenylethylamine, however, Barger and Dale found that methylation increased the activity fivefold. On the other hand, conversion of epinephrine into the corresponding primary amine—removal of the methyl—increased the activity by about 40 per cent (92, 125).

OH
OH
$CH_2CH_2NH_2$

OH
OH
$CH_2CH_2NHCH_3$

Five times more
active than the
primary amine

OH
OH
$CHOHCH_2NH_2$

Norepinephrine

OH
OH
$CHOHCH_2NHCH_3$

Epinephrine

dl-Form 7/10ths as
active as the cor-
responding   pri-
mary amine

Perhaps this anomalous reversal may be attributed to the influence of the secondary alcoholic hydroxyl group in epinephrine. Recently Raymond-Hamet (126) reported another significant pharmacological difference between epinephrine and its corresponding "nor" or primary amino compound. While the pressor effect of epinephrine may be reversed with yohimbine, this drug does not affect the action of norepinephrine.

In the case of the nonphenolic compounds the trend appears more consistent. Chen, Wu and Henriksen, as already stated (124), found phenylethanolamine, $C_6H_5CHOHCH_2NH_2$, much more active than phenylethanolmethylamine, $C_6H_5CHOHCH_2-NHCH_3$; the former is also the more toxic. However, in all the other compounds, the primary amine was found to be not only a

more potent pressor, but also much less toxic. *dl*-Phenylpropanolamine, $C_6H_5CHOHCH(NH_2)CH_3$, is about 20 per cent more active than is *dl*-ephedrine and the toxicity is approximately 15 per cent less. Hartung and Munch (127) found phenylpropanolamine to be as active as *l*-ephedrine and to produce in substantially every respect the characteristic responses of ephedrine; it is active after oral administration, given intravenously to dogs it produces a prolonged rise in blood pressure, and successive doses become less effective. Hasama (118) has published a tracing showing the effect of intravenous administration of phenylpropanolamine to a rabbit; the blood pressure curve showed a rise of comparatively short duration and in general resembled that obtained with phenylethanolamine. Such a result may be caused by an idiosyncracy of the test animal, or perhaps it is a characteristic response of the rabbit to this particular compound. Ehrhart (159) and Schaumann (160) report that in the ephedrine series and phenolic derivatives the primary bases are more strongly active than the corresponding methylamino compounds.

The effect of methylating isoamylamine has already been pointed out; the pressor activity is reduced by about one-half. It is of additional interest to note that the *N*-methylation of histamine reduces its blood pressure lowering capacity to about 1/200th that of histamine itself (120).

An introduction of a second methyl group works to further disadvantage. Hordenine,

$$HO \langle \rangle CH_2CH_2N \begin{matrix} CH_3 \\ \diagup \\ \diagdown \\ CH_3 \end{matrix}$$

Hordenine

the tertiary base corresponding to tyramine, an alkaloid first isolated by Léger (128) from germinating barley and since identified by Späth (129) in cactus species under the name of anhalin, was studied by Camus (130) because of its value in combating diarrhea; it is still distinctly active as a pressor, but it has only 1/10th the activity of tyramine (32, 92).

TABLE 3
The effect of alkylating the amino group

BASE	TOXICITY—M.L.D.	ACTIVITY
(A) ⬡ CH₂CH₂NH₂	40–50 mg./kg. intravenous to rabbits (124)	1/350th as active as epinephrine (32); 0.00002 mole to 2.6 kg. of cat gave 64 mm. Hg (124)
⬡ CH₂CH₂NHCH₃		1/350th as active as epinephrine (32)
(B) ⬡ CHOHCH₂NH₂	80 mg./kg. intravenous to rabbits (124)	0.00002 mole to 2.63 kg. of pithed cat gave 58 mm. Hg (124); 1/350th as active as epinephrine (32)
⬡ CHOHCH₂NHCH₃	100 mg./kg. intravenous to rabbits (124)	0.00002 mole to 2.63 kg. of pithed cat gave 26 mm. Hg (124)
(C) HO-⬡ CH₂CH₂NH₂		1/150th as active as epinephrine (32)
HO-⬡ CH₂CH₂NHCH₃		1/150th as active as epinephrine (32)
HO-⬡ CH₂CH₂N(CH₃)₂	300 mg./kg. intravenous to dogs and guinea pigs; 250 mg./kg. intravenous to rabbits; 2000 mg./kg. subcutaneous to guinea pigs; 2000 mg./kg. oral to dogs (137)	1/700th as active as epinephrine (32); Nicotine-like action (134)

HO⟨⟩CH$_2$CH$_2$(CH$_3$)I		Nicotine-like action (32)
HO⟨⟩CH$_2$CH$_2$NHC$_2$H$_5$		Less than 1/150th as active as epinephrine (32)
(D) HO,HO⟨⟩CH$_2$CH$_2$NH$_2$		1/75th as active as epinephrine (32) 1/65th as active as epinephrine (118a)
HO,HO⟨⟩CH$_2$CH$_2$NHCH$_3$		1/10th as active as epinephrine (32) 1/12th as active as epinephrine (138)
HO,HO⟨⟩CH$_2$CH$_2$NHC$_2$H$_5$		1/43rd as active as epinephrine (32)
(E) C$_6$H$_5$CHOHCHCH$_3$—NH$_2$	175 mg./kg. of hydrochloride intraperitoneal to rats (127) 350 mg./kg. of hydrochloride subcutaneous to rats (137) 600 mg./kg. of hydrochloride subcutaneous to guinea pigs (137) 75 mg./kg. of hydrochloride intravenous to rabbits (137) 70 mg./kg. of hydrochloride intravenous to rabbits (124) 500 mg./kg. of hydrochloride intraperitoneal to dogs (127) 400–500 mg./kg. of sulfate subcutaneous to rabbits (139)	dl-Compound equals l-ephedrine (127) Same as ephedrine (139) dl-Compound 1/80th as strong as epinephrine (124) Strong mydriatic action (140)

TABLE 3—Concluded

BASE	TOXICITY—M.L.D.	ACTIVITY
(E) C₆H₅CHOHCHCH₃* NHCH₃	50 mg./kg. of hydrochloride intravenous to rabbits (127, 141) 350 mg./kg. of hydrochloride subcutaneous to guinea pigs (127) 320 mg./kg. of hydrochloride subcutaneous to rats (142) 400 mg./kg. of hydrochloride subcutaneous to guinea pigs (141) 320–400 mg./kg. of sulfate subcutaneous (140)	dl-Form 1/95th as potent as epinephrine to pithed cat .00001 mole of l-ephedrine gave 75 mm. Hg rise in 2.55 kg. of pithed cat (124)
C₆H₅CHOHCHCH₃ N(CH₃)₂		Much less active than ephedrine (124, 131) 0.00001 mole of l-isomer to 2.55 kg. of pithed cat gave rise of 10 mm. Hg (124)
C₆H₅CHOHCHCH₃ NHC₂H₅	50 mg./kg. intravenous to rabbits (124)	0.00002 mole gave rise of 59 mm. Hg in 2.8 kg. pithed cat
C₆H₅CHOHCHCH₃ ·NHC₃H₇	50 mg./kg. intravenous to rabbits (124)	Gave fall
C₆H₅CHOHCHCH₃ NHCH(CH₃)₂	40–50 mg./kg. intravenous to rabbits (124)	

$C_6H_5CHOHCHCH_3$ $\|$ NHC_4H_9	15 mg./kg. intravenous to rabbits	Gave fall
$C_6H_5CHOHCHCH_3$ $\|$ NHC_5H_{11}	20 mg./kg. intravenous to rabbits (124)	Greater fall (124)
$C_6H_5CHOHCHCH_3$ $\|$ $NHCH_2C_6H_5$	20 mg./kg. intravenous to rabbits (124)	

* For complete table see Chen and Schmidt (140).

l-Methylephedrine,

$$C_6H_5CHOHCHCH_3$$
$$|$$
$$N—CH_3$$
$$|$$
$$CH_3$$

is much less active and appreciably more toxic than ephedrine and it has no longer a mydriatic action (124, 131). Still further methylation—conversion into quaternary ammonium compounds —completely eliminates all pressor activity and confers a nicotine-like action (32, 92, 132, 133). In fact, some of this nicotine-like behavior is present in hordenine even before it is converted into a quaternary ammonium derivative (134).

If the methylamino group is coupled with formaldehyde its physiological action becomes nil (134a).

The use of higher alkyl groups in the place of methyl is a step in the wrong direction. Ethylamine compounds are less desirable than the corresponding methyl derivatives, while propylamine analogs are still less active (92). Of the interesting series of ephedrine-like compounds prepared by Hyde, Browning and Adams (135) it was found (124) that as the alkyl attached to the amino group became larger the activity decreased, the depressant action on the heart became more pronounced, and the toxicity increased. Kanao (136) in a similar series found that if the alkyl group is sufficiently large the compound becomes an anesthetic. Thus $C_6H_5CHOHCH_2NHR$ is anesthetic when R = isobutyl or phenyl. $C_6H_5CHOHCH(CH_3)NHR$ is nonanesthetic when R = ethyl or propyl, but is anesthetic when R = allyl, butyl, isobutyl, isoamyl, benzyl, *p*-aminobenzyl, furfuryl or citral. He also found

$$C_6H_5CH_2CHCH_3$$
$$|$$
$$NHC_3H_7$$

to be anesthetic, whereas the corresponding aminoalcohol,

$$C_6H_5CHOHCHCH_3$$
$$|$$
$$NHC_3H_7$$

is nonanesthetic. This, again, would indicate some peculiar function that may be directly attributed to the alcoholic hydroxyl.

In table 3 will be found a summary of the available quantitative data on the various types of bases under consideration.

The preponderance of evidence thus far, with the very striking exception of 3,4-dihydroxyphenylethylamine, indicates that the primary bases are the most active in their effect on the blood pressure. Conversion into the corresponding secondary amine has a tendency to decrease the pressor activity; in the ephedrine series this becomes more pronounced as the alkyl group grows larger, and soon the sympathomimetic action disappears and a depressant action takes its place; if the alkyl group is allyl, butyl or greater the compound is anesthetic; the toxicity increases as the alkyl group becomes larger. The corresponding tertiary amines are very much less active, while conversion into the the quaternary ammonium derivatives removes all pressor activity and confers nicotine-like action.
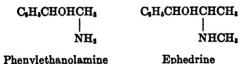
If the primary amines are the most active, why has nature given us secondary bases in ephedrine and epinephrine? Perhaps the activity that the pharmacologist and physiologist are measuring is not the sole indication of clinical desirability or therapeutic efficiency.

VII. INFLUENCE OF CHANGE IN THE LENGTH OF THE SIDE CHAIN

Epinephrine, extensively and variously used in the amelioration or treatment of various human afflictions, has one severe handicap, viz., that it is, as has already been pointed out, ineffective on the blood pressure, except when administered by injection. Hence, when Chen and Schmidt (143) showed that a substantially ephinephrine-like response could be obtained by the oral administration of ephedrine, this plant alkaloid was welcomed into the ranks of the therapeutic agents.

A structural difference between the hormone and the alkaloid that becomes apparent at once is the length of the side chain; the former is a derivative of ethane while the latter is derived from propane. That this is a most significant difference is revealed by the following observations.

Phenylethanolamine, which has been studied by the Council of Chemistry and Pharmacy of the American Medical Association (144, 145), possesses many of the pharmacological actions of ephedrine, except that its action is of much shorter duration; however, it is not active after oral administration. Phenylethanolamine differs from ephedrine by two methyl groups, one on the nitrogen and the other in the side chain.

$$C_6H_5CHOHCH_2 \qquad\qquad C_6H_5CHOHCHCH_3$$
$$| \qquad\qquad\qquad\qquad\qquad |$$
$$NH_2 \qquad\qquad\qquad\qquad NHCH_3$$

Phenylethanolamine   Ephedrine

In order to determine which of the two confers greater oral activity, Piness, Miller and Alles (146) studied two methyl derivatives of β-phenylethylamine; in one the methyl group was substituted on the nitrogen atom, $C_6H_5CH_2CH_2NHCH_3$, and in the other, in the side chain, $C_6H_5CH_2CH(NH_2)CH_3$. They found that the former compound was inactive, whereas the propylamino derivative was very active when taken by mouth; moreover, the duration of its action was very much extended. Chen and co-authors, from their study of ephedrine and its analogs, conclude that it is the third carbon atom in the side chain that confers oral activity. Hartung and Munch (119), from their results with four phenylpropylamines, found that oral activity was conferred when a methyl group was substituted on either carbon atom of the side chain in β-phenylethylamine, both phenyl-2-amino-1-propane and phenyl-1-amino-2-propane being active orally. (See table 2)

If increasing the side chain from two to three carbon atoms confers the desired activity after oral administration, what will further extension do? Strangely enough the whole pressor effect is so far reduced as to be practically negligible. Thus, Chen found phenylbutanolamine, prepared by Tiffeneau (124), to be slightly active in decerebrate cats, and Hartung, Munch, Deckert and Crossley (147) confirmed these findings on anesthetized dogs. On further lengthening of the side chain up to phenyloctanolamine no pressor activity is apparent, the most outstanding change in

physiological activity being a regular increase in toxicity when given intravenously. While the whole series has not yet been thoroughly investigated pharmacologically, phenylhexanolamine is the only member found thus far to exhibit an anomalous behavior; a dose of 10 mg. per kilogram to an anesthetized dog gave first a fall in pressure, which persisted for half an hour, after which the pressure gradually rose and finally went as high above normal as the fall had been below. Schaumann (161) found that if the side chain of epinephrine is increased to four carbon atoms,

$$(HO)_2C_6H_3CHOHCHCH_2CH_3$$
$$|$$
$$NHCH_3$$

the compound is no longer a pressor but has taken on a depressor action.

When phenylethanolamine is compared with the corresponding phenylpropanolamine, one other very marked difference in action appears. An intravenous injection of phenylethanolamine causes a rise in blood pressure that persists for several minutes and then returns to normal; a subsequent administration of the same dose produces an equal effect. Phenylpropanolamine or ephedrine, its N-methylated derivative, given either intravenously or orally, causes a rise in blood pressure that persists for two or more hours, and a subsequent dose produces a response of very much smaller magnitude.

Another significant difference between the two-carbon atom and three-carbon atom side chain derivatives is in their synergistic action, that is, the ability to potentiate the effect of epinephrine. Launoy and Nicolle (148) and Csepai and Doleschall (149) observed that when epinephrine was administered after ephedrine, its action was much greater than normally. The same phenomenon was observed independently by Munch and Hartung (150), and these authors reported that this ability to potentiate the pressor action of epinephrine is characteristic of the derivatives of phenylpropanolamine.

A most striking example of the physiological difference between the two- and three-carbon atom side chains is found in epinephrine and one of its isomers, "norhomoepinephrine."

Epinephrine   Norhomoepinephrine

The latter may be considered as epinephrine in which the *N*-methyl has been shifted to the side chain, giving the latter three carbon atoms. Except for the difference in potencies both produce, after intravenous administration, the same effect on the blood pressure; but, as has already been shown, epinephrine is without any accepted characteristic effect on the blood pressure after oral administration; nor homoepinephrine, on the other hand, was found to produce a very great and prolonged rise in the blood pressure when given by mouth to an anesthetized dog (151).

From all these considerations it is seen that ability to produce a rise in blood pressure is resident in compounds with two or three carbon atoms in the side chain; the ethane derivatives are effective after injection, while the propane derivatives produce an effect of longer duration and possess the added virtue of being potent after oral administration.

By what process is the living organism able to differentiate so readily and characteristically between certain members of an homologous series such as these phenylalkanolamines? The difference is probably more one of kind than of degree; the living tissue seems to be able to distinguish readily where by chemical methods the difference could be determined only with great difficulty. In view of these observations, can pharmacological response be a function only of purely chemical properties?

VIII. THE EFFECT OF HYDROXYL SUBSTITUTION IN THE ÀROMATIC NUCLEUS

Tyramine, hordenine and epinephrine are phenolic substances. It is well-known that the intensity of action of phenolic pressor substances is much greater than that of the corresponding non-phenolic compounds, but the complete rôle of the phenolic func-

tion is not yet known.   Barger and Dale (92) were the first to investigate the rôle of the phenolic hydroxyl group; they compared the three monohydroxy derivatives of β-phenylethylamine and observed that the ortho derivative (I) was no more active
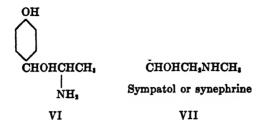
OH
        OH
OH
OH
CH$_2$CH$_2$NH$_2$ CH$_2$CH$_2$NH$_2$ CH$_2$CH$_2$NH$_2$

Tyramine

I   II   III

than the phenylethylamine itself, whereas the meta (II) and para (III) compounds were equally active and about five times more potent than the parent amine.   They found 2,3,4-tri-

OH   OH
OH   OH
OH
COCH$_2$NHCH$_3$ COCH$_2$NHCH$_3$

IV   V

hydroxyphenyl methylaminomethyl ketone (IV) to be less active than 3,4-dihydroxyphenyl methylaminomethyl ketone (V), showing that the introduction of the third, or ortho, phenolic hydroxyl group decreases activity.   Maximum activity was found in 3,4-dihydroxy derivatives, that is, in compounds which had a catechol nucleus.   From these results Barger and Dale concluded that the meta and para hydroxyl groups are of equal influence, and that maximum activity results if both are present.

While Chen, Wu and Henriksen (124) did not work on phenolic compounds, they suggested, largely from the conclusions of Barger and Dale, that the presence of hydroxyl groups in the benzene ring confers "intensity of action" and ventured the prediction that p-hydroxyphenyl-1-amino-2-propanol (VI) might combine the desirable physiological properties of both epinephrine and ephedrine, namely, the intensity of response conferred by the para-hydroxyl, the minimum toxicity and greater activity of the pri-

mary amine, and the duration of action and oral activity conferred by the three-carbon atom side chain.

OH

CHOHCHCH₃
|
NH₂

VI

CHOHCH₂NHCH₃
Sympatol or synephrine

VII

Meanwhile sympatol, or synephrine (VII), described as a stable "adrenaline-like" preparation, received considerable attention. Lasch (152) found it to be relatively nontoxic, with an action on the blood pressure resembling that of epinephrine although of somewhat longer duration and about 1/50th as strong. Hochrein and Keller (153) describe its action as being intermediate between that of epinephrine and ephedrine. Ehrisman (154) reported that he administered it orally to his patients with some evidence of success, and in 1930 the Council on Pharmacy and Chemistry of the American Medical Association (155) accepted synephrine for inclusion in "New and Non-Official Remedies" as a reagent in treating, either *orally* or hypodermically, attacks of hay fever, asthma, coughing, spasms of asthma and pertussis (whooping cough). Ehrisman (154) also reported that sympatol prolonged the effects of novocaine anesthesia two or three times. Tainter (156), in a comprehensive investigation of synephrine and its isomers, found the racemic form to be 1/116th as active a pressor as *l*-epinephrine. As for its oral activity, Stockton, Pace and Tainter (157) found that doses of 0.5–1.5 g. by mouth to patients did not give the desired effects but did induce nausea and vomiting. Nor did these authors find it to have any effect on procaine anesthesia. While the chemist must leave it to the physiologist to settle the question of the oral activity of this compound, nevertheless, from purely structural considerations which have already been discussed, one would expect it to be inactive when given by mouth.

Very recently other phenolic compounds have been prepared.

and a comparison of their pharmacological action will serve best to show the effect of the various phenolic substitutions. The mono-hydroxy compounds include p-sympatol (VII), m-sympatol (VIII) (158), the three monohydroxyephedrines (IX, X and XI) (159, 160, 161) and the three monohydroxyphenylpropanolamines (XI, XII and XIII) (151).
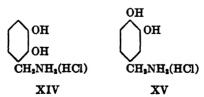
All of the monohydroxyephedrines, when tested on the frog by the Laewen-Trendelenburg method, act like ephedrine itself and possess neither qualitatively or quantitatively the action of epi-nephrine. With ergotamine the meta compound (X) shows a re-versal of effect on the circulation; the para compound is weakened but still active, while the action of the ortho isomer is probably augmented. In cocainized animals the ortho compound shows reversal, the para derivative is nearly inactive, whereas m-hydroxyephedrine shows not only definite potentiation but in-creased duration of action. These characteristic responses after ergotaminization and cocainization indicate that the ortho phenolic compound is most like ephedrine itself; that the meta derivative is least like ephedrine and that it has, in effect, begun to take on the properties ascribed to the catechol nucleus in epi-nephrine (113); and that the para isomer is somewhere interme-diate between the two (161). None of the phenolic compounds exhibited tachyphylaxis, that is, second and third doses showed no

diminishing effects and they also differed from ephedrine and phenylpropanolamine in that larger doses always produced a rise in the blood pressure (151, 161).

The introduction of phenolic hydroxyl groups into phenylpropanolamine tends to cut down the duration of the pressor response. The ortho phenolic hydroxyl serves to increase the toxicity almost twofold and probably to decrease the pressor potency; the meta hydroxyl (XII) increases the activity about threefold and the toxicity by more than four times; the para phenolic function (VI) increases the activity and decreases the toxicity (151).

In modifying any circulatory effects the ortho phenols are least active, which is in agreement with the earlier findings of Barger and Dale (92). However, contrary to these pioneers the meta and para hydroxyl groups do not have equal or identical effects; m-hydroxyephedrine (X) is a stronger pressor than the para isomer (IX) (159, 160, 161); m-hydroxyphenylpropanolamine (XII) is at least twice as strong as p-hydroxyphenylpropanolamine (VI) (151); and Kuschinsky (158) has found that m-sympatol (VIII) is five times as active as is l-p-sympatol (VII). Hence, it is evident that most of the intensifying effect of epinephrine is conferred by the meta phenolic hydroxyl.

The simultaneous substitution of two hydroxyl groups in the aromatic nucleus gives a series of which epinephrine is a member. One may readily see what combinations are possible.

No record was found of a β-phenylethylamine derivative with the two hydroxyl groups in the ortho- and meta-positions. However, Tiffeneau (162) did find that 2,3-dihydroxybenzylamine (XIV) was more effective in increasing the rate and strength of

OH
OH
CH₂NH₂(HCl)

XIV

OH
OH
CH₂NH₂(HCl)

XV
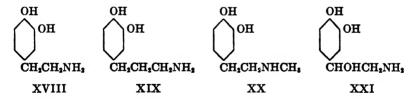
the beat of the isolated rabbit heart, but that 3,4-dihydroxybenzylamine (XV) produced a stronger vasoconstriction and a greater rise in blood pressure.

References to but two 2,4-dihydroxy derivatives were found. Boruttau (163) found that 2,4-dihydroxyphenylethanolamine (XVI)

OH OH

$\bigcirc$OH $\bigcirc$OH

CHOHCH$_2$NH$_2$ CHOHCHCH$_3$

NH$_2$

XVI XVII

had but little effect on the circulation, even in large doses. 2,4-Dihydroxyphenylpropanolamine (not analytically pure) was equally inactive, and at larger doses gave a depression of the blood pressure (151).
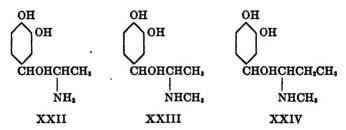
The 3,4-dihydroxy compounds are the best known. The compounds belonging to this class are considered by Tainter (113) as true sympathicotropic reagents, compounds which produce their effect through the sympathetic nervous system. The known catechol derivatives of this type other than epinephrine are discussed below.

3,4-Dihydroxyphenylethylamine (XVIII) is 1/75th to 1/65th as active as epinephrine (32, 118a).

OH OH OH OH

$\bigcirc$OH $\bigcirc$OH $\bigcirc$OH $\bigcirc$OH

CH$_2$CH$_2$NH$_2$ CH$_2$CH$_2$CH$_2$NH$_2$ CH$_2$CH$_2$NHCH$_3$ CHOHCH$_2$NH$_2$

XVIII XIX XX XXI

3,4-Dihydroxyphenylpropylamine (XIX) is, as one might expect from the fact that three carbon atoms separate the amino group and the nucleus, appreciably weaker, that is, it is about one-third as active as the ethylamine homolog (118a). 3,4-Dihydroxyphenylethylmethylamine (XX) may be obtained from papaverine or laudanosine (164, 165) and under the name of epinine it is used as an epinephrine substitute; it is about 1/12th as active as l-epinephrine (138).

3,4-Dihydroxyphenylethanolamine (XXI), also known as arternol, is described as giving substantially all the characteristic epinephrine reactions.   However, being a primary amine, it is more active; Barger (92) and Schultz (125) found it to be about 10/7ths more active and Tiffeneau (166) found it to be twice as active as epinephrine.   Raymond-Hamet (167) observed that if this compound is injected first and followed by epinephrine the latter is the weaker, but if the epinephrine is given first, then it is the stronger.   This author also observed that a yohimbinized animal giving a reversal of action to epinephrine still gives a positive response to the same dose of 3,4-dihydroxyphenyletha-nolamine (XXI).   Tainter (167a) found that dl-3,4-dihydroxy-phenylethanolamine has 80 per cent of the activity of l-epi-nephrine as a circulatory stimulant in cats and that it possesses a therapeutic margin (ratio of toxic dose to effective dose) three times as great.

3,4-Dihydroxyphenylpropanolamine (XXII), an isomer of

epinephrine, has been known for some time.   It was first prepared in the following way.   α-Phthalimidopropionyl chloride was condensed, by means of the Friedel-Crafts reaction, with the dimethyl ether of catechol (168); the resulting ketone was treated with hydrochloric acid, under proper conditions, to yield 3,4-dihydroxyphenyl α-aminoethyl ketone, which after reduction gave the desired aminoalcohol.   A simpler synthesis recently developed consists of the catalytic reduction of 3,4-dihydroxy-phenyl α-oximinoethyl ketone (151).   Kanao (172) prepared the compound with the two phenolic hydroxyl groups acetylated. The hydrochloride is very hygroscopic and can be dried only with the greatest difficulty.   Resolution with d-tartaric acid (169)

gave a levo compound two to three times more potent than the racemic mixture.

Tiffeneau (170) found the l-isomer to be thirty times as active as the d-compound and from 60 to 75 per cent as active as l-epinephrine (171). However, since this compound possesses two asymmetric carbon atoms it is, like ephedrine, theoretically capable of existing in four optically active and two racemic forms; these have not as yet been reported. Bierry, Rathery and Leving (173) found norhomoepinephrine to have hyperglycemic actions resembling those of epinephrine itself. ´ Working with the racemic compound as synthesized, Hartung and Munch (151) found that when it was injected intravenously into rabbits, its toxicity was less than 1/100th as great as that of epinephrine and that qualitatively it is indistinguishable from epinephrine in its effect on the circulatory system; however, it possesses a weaker action, being about 1/12th as potent as l-epinephrine. Schaumann (160) reports it to be 1/6th to 1/3rd as active as l-epinephrine. Since this compound produces the characteristic epinephrine responses and structurally contains the three-carbon atom side chain, it is of particular interest to note that it produces a rise in the blood pressure when given orally. A dog receiving a dose of 100 mg. per kilogram by mouth showed an effect within fifteen seconds and after ninety seconds the blood pressure rose so high that the animal died from cardiac failure. A second animal receiving 1 mg. per kilogram gave, within a minute, evidence of a blood pressure rise, reached a maximum effect in 25 to 30 minutes and the pressure remained at an elevated level for at least two and one-half hours. Two subsequent oral doses both produced rises (151).

Here is an excellent illustration of the fact that the effect produced by substituting groups in a physiologically active molecule is nót necessarily additive, or cumulative, but that their effects may be reciprocally modifying. It has already been shown that in compounds like ephedrine and phenylpropanolamine the duration of action and oral activity are conferred by the third carbon atom in the aliphatic side chain, and that the second and third doses of the same agent produce a much decreased rise in blood pressure. When the meta or para phenolic function was introduced there was a marked diminution in the duration of action,

and second and third doses showed no diminishing effect; simultaneous introduction of the meta and para hydroxyl groups completely neutralizes this duration but apparently has left the oral potency unimpaired.

3,4-Dihydroxyephedrine (XXIII) also gives many of the characteristic epinephrine reactions. Being a secondary amine it is weaker and more toxic than the corresponding primary amine; smaller doses give a fall and larger doses a rise in blood pressure. The substance is definitely sympathicotropic (161).

3,4-Dihydroxyphenyl-1-methylamino-2-butanol-1 (XXIV), containing four carbon atoms in the side chain, is not only weaker than its lower homolog (XXIII), but shows a depressing effect on the blood pressure (161).

While the effect of phenolic substitution on the circulation is readily measured and clearly apparent, it is not improbable that such substitution also modifies the mechanism through which the physiological reaction is produced. Thus, epinephrine is generally accepted as being "sympathicotropic," for all the evidence indicates that its physiological effects are the result of stimulation of the sympathetic system. This sympathicotropic action is ascribed by Tainter (113) to the catechol nucleus, for he found that the pressor effects of the various optical isomers of adrenaline, epinine, adrenalone and even catechol itself were augmented by cocainization and reversed by ergotaminization. Ephedrine, on the other hand, behaves quite differently; ergotamine produces no reversal (174), large doses act directly on the para-sympathetic system (175), and in cocainized animals the effect of ephedrine may be absent or greatly reduced (97, 99). Whether these and other phenomena are to be interpreted as indicative of a purely musculotropic action (99), a predominantly sympathicotropic action (98), or a possible combination of the two is a question to be answered by those better qualified than the chemist. In any event there is an abundance of evidence to indicate that ephedrine is quite different in its mode of action from epinephrine (118a, 176, 177, 178), and perhaps one may not unreasonably expect that in some way there is, as Schaumann suggests (161), a gradual change from a distinctly ephedrine-like reaction to an epinephrine-like reaction as the substitution of a single phenolic

hydroxyl group is shifted from ortho to para to meta, the simultaneous introduction of both the meta and para hydroxyls conferring sympathicotropic reaction.   In any event, all of the compounds which structurally bridge the gap between epinephrine and ephedrine are now available, and further study of their pharmacological properties will continue to throw more light on the physiological variation as one gradually proceeds from one compound to another.

Another difference between the nonphenolic and phenolic compounds, a difference not so well established is in the glycemic action.   The decided hyperglycemic action of epinephrine has been considered one of its most characteristic physiological properties since Blum (179) first observed that suprarenal extracts given subcutaneously produced glycosuria.   Its isomer, nor-homoepinephrine, possesses a similar definite hyperglycemic action (173).   Synephrine injected subcutaneously into rabbits also produces a great rise in the blood sugar (152, 180).   While ephedrine is reported to have a hyperglycemic effect, it is so only in doses much larger, about 20 mg. per kilogram or more, than are necessary to influence the blood pressure (181, 182, 183); in fact, Nitzescu (184) suggested that the hyperglycemic action of ephedrine is a result of induced increased epinephrine secretion.   If ephedrine is administered intravenously during full digestion smaller doses, 0.5 to 3 mg. per kilogram, are reported to be hyperglycemic (184).   Others have found the hyperglycemic action to be comparatively low or even insignificant (185, 186).

Hence it is seen that the phenolic hydroxyl groups, especially those in the meta and para positions, contribute very specifically to the potency of the molecule and probably also modify very materially the mechanism of the physiological response.
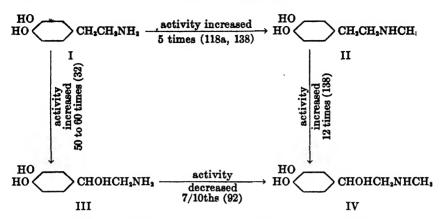
IX. THE EFFECT OF HYDROXYL IN THE SIDE CHAIN

The significance of the alcoholic hydroxyl group in epinephrine and ephedrine does not appear to be completely defined.   We have already seen that when 3,4-dihydroxyphenylethylamine (I) is methylated the pressor activity is increased fivefold, whereas the N-methylation of 3,4-dihydroxyphenylethylamine (III) decreases the activity in the ratio of 7:10 (92).

TABLE 4

Effect of alcoholic hydroxyl group in side chain

AMINE	TOXICITY—M.L.D.	PHYSIOLOGICAL ACTIVITY
(A) $C_6H_5CH_2CH_2NH_2$	450 mg./kg. of hydrochloride subcutaneous to rats (119) 60 mg./kg. of hydrochloride intravenous to rabbits (119) 40–50 mg./kg. of hydrochloride intravenous to rabbits (124) 200–250 mg./kg. of hydrochloride subcutaneous to guinea pigs (187)	1 mg./kg. to dog gave good rise that persisted 20 min. (119) Doses larger than 1 mg./kg. to rabbits produced fall (118)
$C_6H_5CHOHCH_2NH_2$	80 mg./kg. of hydrochloride intravenous to rabbits (124) 90 mg./kg. of hydrochloride intravenous to rabbits (147) 1000 mg./kg. of hydrochloride subcutaneous to guinea pigs (147, 187) 30 mg./kg. of hydrochloride intravenous to rabbits (187)	Pressor activity equals that of ephedrine (187) 0.2 cc. of $M/40$ solution to decerebrate cat produced rise in blood pressure of 58 mm. Hg (124) 0.5–1.2 mg. intravenous to rabbits, cats and dogs caused rise in blood pressure of 10 to 280 per cent (188)
(B) $C_6H_5CH_2CH(NH_2)CH_3$	25 mg./kg. of hydrochloride intravenous to rabbits (119) 25 mg./kg. of hydrochloride subcutaneous to rats (119)	1 mg./kg. gave rise equal to that of $C_6H_5CH_2CH_2NH_2$; effect persisted longer (119)
$C_6H_5CHOHCH(NH_2)CH_3$	75–90 mg./kg. of hydrochloride intravenous to rabbits (119)	Equals ephedrine (119)

	Structure		Activity
(C)	HO⟨⟩CH₂CH₂NHCH₃		1/70th as active as dl-epinephrine (32)
	HO⟨⟩CHOHCH₂NHCH₃	50 mg./kg. of hydrochloride intravenous to rabbits (152) 500 mg./kg. of hydrochloride subcutaneous to rats (152)	1/58th as active as dl-epinephrine(189) 0.2 cc. of M/20 solution to decerebrate cat produced rise of 45 mm. Hg (124)
(D)	HO, HO⟨⟩CH₂CH₂NHCH₃		1/12th as active as l-epinephrine (188)
	HO, HO⟨⟩CHOHCH₂NHCH₃		Activity of epinephrine previously given
(E)	HO, HO⟨⟩CH₂CH₂NH₂		1/50th as active as corresponding aminoalcohol derivative (32a) 1/65th as active as epinephrine (118a)
	HO, HO⟨⟩CHOHCH₂NH₂		50 times more active than simple amine (32)

This reversal of effect is obviously caused by the presence of the alcoholic function. It is also seen that the introduction of an alcoholic group increases tremendously the pressor potency, fifty or more times in the primary amine and twelve times in the secondary amine. These data serve as an excellent example that the compounded effect of the simultaneous introduction of two substituents into a physiologically active molecule may be reciprocally modifying; one cannot always rely on a summation of their individual effects.

Another outstanding difference between epinephrine and its desoxy compound, epinine (II), is the fact that the average duration of blood pressure rise for epinine is about twice as long as for *l*-epinephrine (138). There is insufficient evidence as yet to know whether the introduction of the alcoholic group generally increases the pressor potency (124).

Comparing β-phenylethylamine with the corresponding amino-alcohol, phenylethanolamine, Hasama (118) found the following:

$C_6H_5CH_2CH_2NH_2$	$C_6H_5CHOHCH_2NH_2$
1. Small doses give blood pressure rise. Doses larger than 1 mg. per kilogram cause depression.	1. Always produces rise..
2. No parallelism between dose and degree of response.	2. Degree of rise parallels the dose.
3. Minimum effective dose is 0.05 mg. per kilogram intravenous to rabbits.	3. Minimum effective dose is 0.1 mg. per kilogram.
4. Blood pressure curve rises gradually and falls sharply.	4. Blood pressure curve rises sharply and falls sharply.

Comparing the curves obtained from β-phenylethylamine and tyramine with those obtained with the corresponding alcohols it is seen that the presence of the hydroxyl group in the side chain makes the compound reach its maximum effect in much shorter time.

In the propane series Hartung and Munch (119) found that the introduction of the alcoholic group on the carbon atom adjacent to the aromatic nucleus in phenyl-1-amino-2-propane, that is, going from $C_6H_5CH_2CH(NH_2)CH_3$ to $C_6H_5CHOHCH-(NH_2)CH_3$, decreased the toxicity from 25 mg. per kilogram to 350 mg. per kilogram subcutaneously injected into rats, and from 25 mg. per kilogram to 75 mg. per kilogram intravenously administered to rabbits, a decrease from three- to sixteen-fold, depending on the method of determination. The effect on the relative pressor potency has not yet been determined.

The mydriatic effect of these compounds may, in part, be ascribed to the alcoholic group, for compounds without this group are not mydriatic (124).

The results thus far available on the rôle of the hydroxyl group in the side chain are given in table 4.

A study of this table indicates that in.general the alcoholic hydroxyl attached to the carbon bearing the aromatic group serves to detoxicate, at least in part, and to augment the pressor activity. Whether it also affects the mode of action has not been determined, nor is there any evidence to indicate what may be expected if the hydroxyl group is shifted to other positions in the side chain.
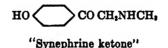
X. AMINOKETONES

If the secondary alcoholic group in the aminoalcohols, is oxidized, the corresponding ketonic derivative is obtained. While numerous ketones of this type are known, relatively few of them have been studied pharmacodynamically. The ketone corresponding to epinephrine, adrenalone, was the first known (23) and this was found to possess an action qualitatively like that of epinephrine. It has been introduced as a clinical reagent under the name of "stryphnon," but apparently is not being widely used as an epinephrine substitute. In its action it is much weaker;

Barger and Dale (32, 92) found it less than 1/20th as active as
dl-epinephrine. Others have found the ratio to vary from
1:200 to 1:300 for adrenalone and l-epinephrine (190, 191).
Tainter (138) was unable to establish a definite figure, since
this ratio varied from 1:100 to 1:200. However, the duration of
the pressure rise was approximately three times that obtained
with l-epinephrine.
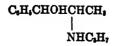
If the N-methyl is removed from adrenalone the activity is
increased somewhat—quite as might be expected. If, however,
it is replaced by an ethyl group the activity is increased by more
than half, whereas substitution by a propyl radical decreases
activity to about 1/5th (32, 92). In the light of the general
effects of alkylating the amino nitrogen it looks as if the ethyl
homolog of adrenalone is overrated. Should the value it now
has be substantiated by future findings, it will be an excellent
example of the reversal of a trend.

Barger and Dale also examined other ketones. Their effects
were not extensively investigated but in general their activity was
markedly less than that of the corresponding alcoholic derivatives.

$$HO \langle \bigcirc \rangle CO\ CH_2NHCH_3$$

"Synephrine ketone"

A ketone that does deserve more than passing notice is that
corresponding to synephrine (sympatol). Tainter and Seidenfeld
(189) found this compound to behave quite differently from the
corresponding aminoalcohol. The first injection usually gave
a predominantly pressor response, although the curve was irregu-
lar because of the coincident depression. The second dose always
produced a fall in blood pressure. This depression was induced
by the large dose required, 10 to 20 mg. per kilogram intrave-
nously, to produce a rise in blood pressure.

Chen, Wu and Henriksen (124) studied the action of phenyl-
propanolpropylamine,

$$C_6H_5CHOHCHCH_3$$
$$|$$
$$NHC_3H_7$$

and its corresponding ketone,

$$C_6H_5COCHCH_3$$
$$|$$
$$NHC_3H_7$$

and found both to cause a fall in blood pressure. In the latter compound this effect is to be attributed perhaps more to the influence of the large alkyl group substituted on the amino group than to the ketonic structure.

While our knowledge of pharmacodynamic relationships between the aminoketones and the corresponding aminoalcohols leaves much to be desired, all the evidence now available shows that the ketones are much less active and results with them are less capable of being reproduced.

XI. OTHER SUBSTITUTIONS IN THE AROMATIC NUCLEUS

The compounds thus far considered have been phenyl or hydroxyphenyl derivatives. Recently compounds with other substituents in the aromatic nucleus have been studied with very interesting results.

Hartung and Munch (127, 147) found that a methyl group substituted in the para position of phenylpropanolamine, namely a new isomer of ephedrine, decreases the activity to about 3/5ths and increases the toxicity about threefold. In an analogous manner the introduction of a methyl group into the para position of phenylbutanolamine very greatly increases its lethal effect. The greater toxicity of the p-tolyl derivatives as compared to the corresponding phenyl compounds is further shown by the work of de Burnaga Sanchez (192), who found p-methylephedrine to be about 20 per cent more toxic than ephedrine, and at the same time less active. A methyl group substituted in the meta position of phenylpropanolamine (I) seems to increase the toxicity as much as does a methyl group in the para position and probably also decreases the pressor activity (192a).

The introduction of a methyl group into phenolic derivatives of phenylpropanolamines produced unexpected results. The 3-methyl-4-hydroxy derivative (II)

$$\begin{array}{ccc} \text{CH}_3 & \text{OH} & \text{CH}_3 \\ & \overset{\text{CH}_3}{\bigcirc} & \overset{\text{OH}}{\bigcirc} \\ \text{CHOHCHCH}_3 & \text{CHOHCHCH}_3 & \text{CHOHCHCH}_3 \\ | & | & | \\ \text{NH}_2 & \text{NH}_2 & \text{NH}_2 \\ \text{I} & \text{II} & \text{III} \end{array}$$

is about twice as active and almost four times as toxic as phenyl-propanolamine; that is, it possesses the increased activity conferred by the phenolic hydroxyl and the toxicity of the *m*-methyl group. Its isomer, 3-hydroxy-4-methylphenylpropanolamine (III), while equally as active was very much less toxic, less toxic in fact than phenylpropanolamine itself, which is contrary to anticipation, since the substitution of either the *p*-methyl or the *m*-hydroxyl alone increases the toxicity severalfold. It is most unexpected that their simultaneous introduction should counteract mutually their individual toxic effects (151).

Several compounds with other alkyl substitutions in the phenyl nucleus have been prepared. Manske and Johnson (192b) synthesized *p*-ethylphenylpropanolethylamine,

$$\text{C}_2\text{H}_5 \cdot \text{C}_6\text{H}_4\text{CHOHCH(NHC}_2\text{H}_5)\text{ CH}_3$$

and 2,5-dimethylphenylpropanolethylamine,

$$2,5\text{-(CH}_3)_2\text{C}_6\text{H}_3\text{CHOHCH(NHC}_2\text{H}_5)\text{ CH}_3$$

but they gave no indication of the physiological activity of these compounds. Ehrhart (159), however, stated that the *p*-ethyl and *p*-butyl derivatives of ephedrine are strongly toxic and exhibit no circulatory effects.

Results thus far would seem to indicate that alkyl substitution in the aromatic nucleus of ephedrine compounds produces effects analogous to similar substitution on the amino nitrogen.

Barger (32) prepared a series of para substituted β-phenyl-ethylamines in which the substituent groups were —COOH, —COOC$_2$H$_5$, —NO$_2$, —NH$_2$ and Cl. The pressor activity of these compounds was determined by Tainter (118a). The *p*-COOH derivative was practically inactive, but its ethyl ester

was distinctly active, about 1/900th as active as epinephrine. The p-NO$_2$ compound gave first a small transitory depression which was followed by a marked rise that lasted for about five minutes; it is 1/823rd as strong as epinephrine. p-Chlorophenylethylamine is 1/368th as active as epinephrine and about half as active as tyramine.   This would indicate that the effect of the chlorine in the aromatic nucleus compares favorably with that of a phenolic hydroxyl.   All of these compounds are desensitized by cocaine and, hence, are characterized as pseudosympathicotropic.

Among other compounds of this general type which have been prepared are dichlorotyramine (V), dibromotyramine (IV) (193) and diiodotyramine (VI); the action of the latter compound has been found similar to that of the thyroid (194); a piperonal (VII) and methoxypiperonal derivative (VIII), a veratrol compound (IX) and the methyl ether of tyramine (X) (195, 208a); 2,4-dinitrophenylethylamine (XI) (196), β-phenyl-β-chloroethylamine (XII) (197), phenyl-1-chloro-1-amino-2-propane (119) and the methylene ether of epinephrine (XIV) (198).

$$
\begin{array}{ccc}
\text{O—CH}_3 & \text{OCH}_3 & \text{OCH}_3 \\
\bigodot\text{O} & \text{CH}_3\text{O}\bigodot\text{OCH}_3 & \text{CH}_3\text{O}\bigodot\text{OCH}_3 \\
\text{CHOHCH}_2\text{NHCH}_3 & \text{CH}_2\text{CH}_2\text{NH}_2 & \text{CH}_2\text{CH}_2\text{NH}_2 \\
 & \text{Mescaline} & \\
\text{XIV} & \text{XV} & \text{XVI}
\end{array}
$$

However, except for XIII, which is an active pressor (199), no reference was found as to their activity.

Another β-phenylethylamine substance that is of unusual interest is its 3,4,5-trimethoxyderivative (XV), known also as mescaline. Its relationship to the pressor compounds depends on its structure rather than on its physiological behavior. It is a natural product and is one of the six alkaloids found by Heffter (200) in the "button" of *Anhalonium Lewinii*, a cactus species, also known as mescaline; its structure was established by Späth (129). It is responsible, chiefly, for the color visions and religious fervor induced by "peyote" (201, 202), the ceremonial object of a growing religious cult among the Indians of Northern Mexico and Southwestern United States (203, 204, 205). Mescaline has been used with indifferent success in the treatment of psychopathic disturbances (206). Dixon, in examining the effect on the blood pressure (202), found that 0.05 g. of mescaline injected into the veins of a cat gave a fall; after seven and one-half minutes there was a sudden rise to normal and a minute later a sudden rise above normal. Raymond-Hamet (206a) reported mescaline as being practically without any effect on the blood pressure.

Jansen found that if one *m*-methoxyl group in mescaline is shifted to the ortho position (XVI) the stupefying effect is not lost, but the exalted feelings, pleasant color visions and general euphoria are no longer produced (207). For a more comprehensive treatise of the physiological and psychic results obtained with mescaline, the reader is referred to the monograph by Kurt Beringer on "der Mezkalinrausch" (208).

In the arylpropanolamine series several methoxy compounds are known. Hartung, Munch, Miller and Crossley (151) pre-

pared three methoxy substituted derivatives of phenylpropanol-amine, and found that the orthoderivative (XVII) was as active but more than twice as toxic as phenylpropanolamine; the intro-

$$OCH_3 \qquad\qquad OCH_3 \qquad\qquad OCH_3$$

OCH$_3$
CHOHCHCH$_3$ ·   CHOHCHCH$_3$ OCH$_3$ CHOHCHCH$_3$
| | |
NH$_2$ NH$_2$ ·   NH$_2$

XVII   XVIII   XIX

duction of the p-methoxy group into phenylpropanolamine (XVIII) increased the toxicity twofold and reduced the pressor activity to about one-half; 2,4-dimethoxyphenylpropanolamine (XIX) was about as active but more than three times as toxic as phenylpropanolamine. Koller (208b) found p-methoxyephedrine to act like ephedrine but to be weaker in action, and that m-methoxy-p-hydroxyephedrine (XXI), when injected into rabbits, produced an indefinite effect on the circulation and a definite slowing of the respiration.

OCH$_3$ CH$_3$ · NH$_2$

OCH$_3$

CHOHCHCH$_3$ CHOHCHCH$_3$ CHOHCHCH$_3$
| | |
NHCH$_3$ NHCH$_3$ NHCH$_3$

XX   XXI   XXII

Very recently the synthesis of p-aminoephedrine (XXII) was announced (208c). This compound, introduced as "ephetonal," is described as being 1/3rd to 1/2 as toxic as ephedrine; it is said to exhibit typical ephedrine-like action on the sympathetic system, but unlike ephedrine it does not exhibit tachyphylaxis nor do large doses produce a depression in the blood pressure (208d).

From the evidence thus far available it appears that the phenyl group in the skeleton of pressor substances may undergo consider-able modification before the circulatory effect is eliminated.

XII. OTHER COMPOUNDS

In addition to the substances thus far discussed, there are various individual compounds which deserve mention because of their general structural relationship to the series.

β-Tetrahydronaphthylamine (I)

$$\begin{array}{c}\text{CH}_2 \\ \text{CHNH}_2 \\ \text{CH}_2 \\ \text{CH}_2\end{array}$$

I

may be considered as a derivative of β-phenylethylamine, of γ-phenylpropylamine or of cyclohexylamine (32). This compound, when administered as the hydrochloride intravenously to a dog or rabbit in doses varying from 10 to 70 mg. per kilogram of body weight, produced curare effect, increase in temperature, and a strong rise in the peripheral blood pressure (209).

N-methylation gave a product that exerted a more marked effect on the blood pressure but decreased the duration effect. A second methyl group on the nitrogen reduced the stimulating effects very markedly and decreased the toxicity, but did not destroy the mydriatic activity. Conversion into the quaternary ammonium salt gave a molecule that exhibited a curare-like action, caused increase in blood pressure and dilated the pupils, but produced no effect in the body temperature (210). The effect of methylation in β-tetrahydronaphthylamine is quite different from that in other pressor compounds.

The activity of β-naphthylethylamine derivatives was investigated by Madinaveitia (211, 212). By comparing the activity of β-phenylethylmethylamine (II) with the methylether of phenylethanolmethylamine (III)

$$\underset{\text{II}}{\text{CH}_2\text{CH}_2\text{NHCH}_3} \qquad \underset{\text{III}}{\underset{\text{OCH}_3}{\overset{\displaystyle \text{CHCH}_2\text{NHCH}_3}{|}}} \qquad \underset{\text{IV}}{\underset{\text{OCH}_3}{\overset{\displaystyle \text{CHCH}_2\text{NHCH}_3}{|}}}$$

he observed that the introduction of the methoxyl group into the side chain did not change the sympathomimetic activity; but if the α-naphthyl nucleus (IV) was substituted for the phenyl, the activity was increased about forty times. Having found the naphthyl compound so active, Madinaveitia compared the activity of four other derivatives (V to VIII).

OH

COCH$_2$NH$_2$(HCl)   CHCH$_2$NH$_2$(HCl)   COCH$_2$NH$_2$(HCl)
                        |
                        OCH$_3$

V   VI   VII

and found that introduction of the hydroxyl group para to the side chain (V) greatly increased the activity and that etherification of the phenolic hydroxyl (VIII) greatly reduced the intensity; the ketone is much less active than the methyl ether of the corresponding alcohol, for 3 mg. of VI produced the same effect as was obtained from 20 mg. of VII.

OCH$_3$

COCH$_2$NH$_2$(HCl)

VIII

Another group of naphthylaminoalcohols was prepared by Fourneau, Tréfouel and Tréfouel (213), which has the general structure

CH$_2$CHOHCH$_2$N

and in which the naphthyl nucleus or the amino group, or both contained substitutes. The aim of these scientists was to determine, if possible, whether the naphthalene nucleus might not be

substituted for the quinoline portion in compounds that possess antimalarial action.

In the search for compounds simpler than plasmochin, Fourneau and Brydowna (214) also prepared p-aminophenyl-1-piperidino-3-propanol-2 (IX) but found it ineffective against malaria in birds.

NH₂

CH₃CHOHCH₂N

$$NH_2$$

$$CH_3CHOHCH_2N \diagup \diagdown$$

IX

The sympathomimetic action of Fourneau's compounds appears not to have been determined, and from structural considerations alone one would expect them to possess little or no such action.

Since the appearance of ephedrine in a major rôle as a clinical reagent, various attempts have been made to synthesize a substance of different structure which would retain all the desired actions and not have the undesirable ones. The results of modifications in the side chain, in the amino group, and of the introduction of a para alkyl group have already been discussed. There are, however, other modifications, less easily classified, which must also be considered.

Dulière (215), prepared various ethers of ephedrine, and found that as the alkyl group attached to the alcoholic hydroxyl became larger, the toxicity increased and the characteristic ephedrine reaction became less pronounced. Some of the corresponding

OH

OH

CHCH₃NHCH₃

OCH₃

X

CH₃CHCHOHCH₃

NHCH₃

XI

CH₃—C—CH₂OH

CH₃NHCH₃

XII

ethers of epinephrine, that is, through the alcoholic hydroxyl, have been prepared by Funk and Freedman (216). It was found (217) that the methyl ether (X) possessed a weaker hyperglycemic action than epinephrine, and that the ethyl ether was still less active.

Fourneau and Barrelet (218) prepared phenyl-4-methylamino-3-butanol-2 (XI) and found it to be much weaker than ephedrine, showing again the effect of going beyond the three-carbon atom side chain. On the other hand, phenyl-2-methyl-2-methylamino-3-propanol-1 (XII), which has a methyl substituted side chain of three carbon atoms, was found to be quite active (219). A dog receiving a dose of 0.2 mg. per kilogram gave a rise of 40 mm. of mercury in the arterial pressure and the effect persisted for some time; larger doses (5 mg. per kilogram) produced only a feeble rise.

It has already been pointed out that the aromatic portion of the molecule may be a substituted phenyl or even a naphthyl group, although each structural modification does affect in some way the physiological response produced. Apparently the aromatic nucleus need not always be strictly hydrocarbon in nature. Hildebrandt reports that thebenine (XIII) (220) has a general reaction toward rabbits like that of 3,4-dihydroxyphenylethanolamine (XIV), and he credits this to the influence of the "Verbindungskette."

XIII   XIV   XV

Kaufmann (221) found quinolinylethanolamine (XV) to act on the blood pressure as does phenylethanolamine. Hasegawa (222), found β-indolethylamine (XVI) to dilate the pupil markedly and that large doses given intravenously gave an initial rise followed by a fall in blood pressure. Seki (222a), working with the α-methyl derivative of β-indolethylamine, found that it produced a rise in blood pressure by vasoconstric-

tion, and that it contracted the uterus and stimulated intestinal movements.

$$\text{XVI} \quad \text{Indole-CH}_2\text{CH}_2\text{NH}_2 \qquad \text{XVII} \quad \text{Furan-CHCHOHCH}\begin{smallmatrix}R\\NH_2\end{smallmatrix}$$

<div align="center">

structure XVI	structure XVII
XVI	**XVII**

</div>

Kanao found the furylamino alcohols (XVII) to possess a mydriatic action, the intensity of which became less as the length of the side chain increased (223). Windaus and Dalmer (223a) found furethylamine to produce only a short-lived fall in blood pressure and that its tetrahydroderivative was without any effect. Hinegardner and Johnson (224) have prepared "thiazole bridges" of epinephrine- and tyramine-like bodies (XVIII)

<div align="center">

structure XVIII	structure XIX
XVIII	**XIX**

</div>

and report that these compounds possess pharmacological interest. Tainter (118a) found thienylethylamine (XIX) to be about as active as the phenyl analog.
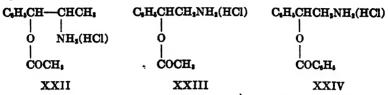
Recently Crook and McElvain (225) prepared some phenylpiperidylcarbinols (XX and XXI)

<div align="center">

C$_6$H$_5$CHOHCH—NH—CH$_2$ CH$_2$—CH$_2$—CH$_2$	C$_6$H$_5$CHOHCH—CH$_2$—NH CH$_2$—CH$_2$—CH$_2$
XX	**XXI**

</div>

which may be considered as analogs of ephedrine, and which are reported to have weak ephedrine-like action.

From a survey of all the compounds considered it would seem that examination of various modifications in the aromatic nucleus promises more positive results than do the changes in the side chain or the amino group.

While the etherification of the alcoholic hydroxyl has been found to affect somewhat adversely the activity of the molecule,

the influence of esterification has been determined only in part. Kester and Munch (226) found that the acetic ester of phenyl-propanolamine (XXII)
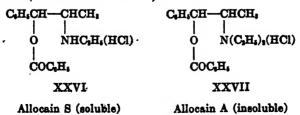
$$C_6H_5CH\text{—}CHCH_3 \qquad C_6H_5CHCH_2NH_2(HCl) \qquad C_6H_5CHCH_2NH_2(HCl)$$

$$\underset{\displaystyle O \quad NH_2(HCl)}{|} \qquad \underset{\displaystyle O}{|} \qquad \underset{\displaystyle O}{|}$$

$$\underset{\displaystyle COCH_3}{|} \qquad \underset{\displaystyle COCH_3}{|} \qquad \underset{\displaystyle COC_6H_5}{|}$$

$$\text{XXII} \qquad\qquad \text{XXIII} \qquad\qquad \text{XXIV}$$

was practically devoid of pressor activity. Wolfheim (197) prepared the acetic acid and benzoic acid esters of phenylethanol-amine (XXIII and XXIV), but gave no pharmacological data concerning them.

The benzoic ester (XXIV) should be of particular interest because of its structural similarity to anesthetics of the procaine type (XV)

$$CH_2CH_2N \Big\langle \begin{array}{l} C_2H_5 \\ C_2H_5 \end{array}$$

$$\underset{\displaystyle O}{|}$$

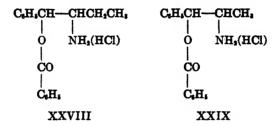$$\underset{\displaystyle CO}{|}$$

NH₂

Procaine

XXV

Would it continue to act as a pressor and at the same time take on anesthetic properties? If so, this would be the first synthetic anesthetic-pressor. Kubota (227) examined higher homologs, the allocains (XXVI and XXVII)

$$C_6H_5CH\text{—}CHCH_3 \qquad\qquad C_6H_5CH\text{—}CHCH_3$$

$$\underset{\displaystyle O \quad NHC_2H_5(HCl)}{|} \qquad \underset{\displaystyle O \quad N(C_2H_5)_2(HCl)}{|}$$

$$\underset{\displaystyle COC_6H_5}{|} \qquad\qquad \underset{\displaystyle COC_6H_5}{|}$$

$$\text{XXVI} \qquad\qquad\qquad \text{XXVII}$$

Allocain S (soluble)   Allocain A (insoluble)

and found them to be very strong as anesthetics, while in the circulatory system, they produced first a fall in the blood pressure and then a rise.   Recently Tiffeneau (228) described the benzoic ester of phenylbutanolamine (XXVIII)

$$C_6H_5CH-CHCH_2CH_3$$
$$\quad\;\; |\qquad\quad |$$
$$\quad\;\; O\quad\; NH_2(HCl)$$
$$\quad\;\; |$$
$$\quad\;\; CO$$
$$\quad\;\; |$$
$$\quad\;\; C_6H_5$$

$$C_6H_5CH-CHCH_3$$
$$\quad\;\; |\qquad\quad |$$
$$\quad\;\; O\quad\; NH_2(HCl)$$
$$\quad\;\; |$$
$$\quad\;\; CO$$
$$\quad\;\; |$$
$$\quad\;\; C_6H_5$$

XXVIII   XXIX

as an anesthetic approaching cocaine in potency.   Now, if generalizations are of any value to the research worker one may reasonably argue that while Kubota's allocaines (XXVI and XXVII), with alkyl substitution on the nitrogen that decreases the potency of the pressor portion of the molecule, and Tiffeneau's compound, the ester of a substance which by itself possesses a structure that weakens the activity, may be very potent anesthetics, they would probably not possess, at the same time, marked pressor powers, unless esterification should serve to intensify them.   On the other hand, phenylethanolamine and phenylpropanolamine are highly active, and if esterification decreases the pressor activity, their benzoic esters (XXIV and XXIX) should *a priori* preserve this potency to a maximum, if at all. To determine this experimentally Hartung, Munch and Kester (229) investigated the ester of phenylpropanolamine (XXIX) and found that a dog receiving an intravenous dose of 10 mg. per kilogram gave a maximum rise in blood pressure of 26 mm. of mercury, and that the rise continued for more than three hours. When tested on the rabbit's eye it produced an anesthetic effect several times greater than that of novocaine.   This is, so far as these authors are aware, the first compound with a demonstrated simultaneous pressor and anesthetic action.   There is every reason to believe that Wolfheim's ester (XXIV) should act in a similar manner.

XIII. OPTICAL ISOMERISM

Up to this point emphasis has been placed on molecular modifications involving the introduction, elimination, or shift in the point of attachment of an element or organic radical. In addition to such changes there is another of a quite different nature that has its own mysterious influence on physiological reactivity, and which is very germane to the whole subject under discussion, namely, optical isomerism. Optical phenomena, ever since their discovery by Pasteur, have been very fascinating to the chemist, particularly their influence on the pharmacological reaction.

It was early noticed that natural epinephrine was levorotatory (21) and that the synthetic, i.e., racemic, compound was very much less active. Its resolution into the optically active components gave a levo form with an activity equal to that of the natural product. Cushny (230) found that natural epinephrine was twice as strong as the synthetic, optically inactive compound. Abderhalden and Müller (231) found the levo form fifteen times more active than the dextro form; Cushny (232) found the ratio to be $1:12$. Others have checked it and found the ratio of $l:dl:d$ to be $1:1/2:1/12$ to $1/40$ (113, 191, 233, 234, 235, 236, 237). Ishiwara (84) reported that by the Trendelenburg method there is practically no difference between the activity of the racemic and the optically active forms. Richaud is quoted by Bierry, Rathery and Leving (173) as saying that the difference in the hypertensive power of l- and dl-epinephrine disappears as the magnitude of the dose increases, that is, doses of 0.04 to 0.05 mg. produce sensibly the same effects.

The levo isomer is not only more active but it is also more toxic than d-epinephrine—from six to twenty times, depending on the method of determination (191, 232). Mice, pretreated with d-epinephrine, develop a tolerance for ten or more times the normal lethal dose toward the l-variety (238, 239), but such immunity is only temporary (240). It has even been reported (241) that the intravenous injection of d-epinephrine to cats and dogs would render them nonresponsive to larger doses of l-epinephrine.

Tainter (113), working with anesthetized cats, found the ratio

of activity for the l: dl: d isomers to be 1: $1/2$: $1/30$. In addition Tainter pointed out that the three isomers have their own characteristic effects in the duration of the pressor response. By employing doses which produced moderate and approximately equal elevation of the blood pressure, he observed that the rise following l-epinephrine lasted for an average of 1.5 minutes, while from the racemic mixture the average was 2.5 minutes, but the d-epinephrine gave a much longer rise, an average of 4.7 minutes. Tainter believes that this longer effect "would be favorable to the practical use of the dextro compound, since the extremely evanescent action of l-epinephrine has always been a drawback"; he also feels that this difference indicates "somewhat of the selectivity of the pressor responses,—a selective difference in protoplasmic reaction, which apparently was conditioned on a difference in configuration of the drug-molecule."

The Pharmacopoeia recognizes only l-epinephrine. Whether or not correspondingly larger doses of the racemic mixture, or even of the d-isomer, should not be of equal therapeutic merit apparently has never been determined.

From the laboratory of Tainter and Seidenfeld has come another valuable contribution to our knowledge of the influence of optical isomerism on physiological reaction. These investigators (189) found the ratio of activity for the synephrine isomers to vary in the following manner: l: dl: d = 1: $1/2$: $1/60$. Here also the d-synephrine gave a longer duration of blood pressure rise, a median of three minutes as compared to two minutes for both the l- and dl-compounds. When the effect of the three synephrine isomers was studied on perfused rabbit ears, unexpected results were obtained. Doses of 0.5 to 2.0 mg. of l-synephrine caused prompt vasoconstriction; the d-isomer even up to 10 mg. usually had no effect or caused dilatation; the racemic substances in doses ranging from 1 to 50 mg. never did produce constriction. Since the racemic mixture contains equal parts of the two optically active components it appears that the d-isomer is not only inactive but is also able to suppress the rather powerful vasoconstrictor action of the l-isomer. This is indeed an unusual example of antagonism.

The levorotatory isomer of the hypertensive amines has usually been found to be the most active. Thus *l*-norhomoepinephrine is two or three times as active as the racemic mixture and thirty times as active as the *d*-compound (169, 171). Of all the synthetic ephedrine analogs which have been resolved, the levorotatory form has always been found to be the most active (208b, 218). Of the pseudoephedrines, however, the *d*-form is the more active.

Ephedrine exists in the form of known optical isomers, but since it contains two asymmetric carbons

$$\underset{\text{OH} \quad \text{NHCH}_3}{\overset{\text{H} \quad \text{H}}{C_6H_5C - C - CH_3}}$$

there are possible six isomers, four optically active and two racemic mixtures, namely *d*- and *l*-ephedrine and *d*- and *l*-pseudo-ephedrine and the respective racemic mixtures.
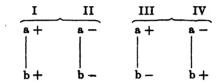
The constants for the six isomers are given in table 5.

TABLE 5

The isomers of ephedrine

ISOMER	MELTING POINT OF THE HYDROCHLORIDE	ROTATION OF HYDROCHLORIDE	
	°C.		
l-Ephedrine	214–216	$[d]_D^{25} = -35°$ (124)	$[M]_D^{20} = -72°$ (242)
d-Ephedrine	213	$[d]_D^{20} = +35°$	$[M]_D^{20} = +72°$
dl-Ephedrine	187	— —	— —
l-ψ-Ephedrine	178–179	$[d]_D^{24} = -62.5°$	$[M]_D^{20} = -125°$
d-ψ-Ephedrine	180.5	$[d]_D^{25} = +62.5°$	$[M]_D^{20} = +125°$
dl-ψ-Ephedrine	163	— —	— —

In nature the alkaloid is found as *l*-ephedrine, *d*-pseudo-ephedrine, or as a mixture of the two (243). Ernst Schmidt (244) early demonstrated the geometric isomerism between the two products from the ease with which they were interconvertible.

Gadamer (245) believed that such isomerization was caused by an "Umklappen," a shift of the hydroxyl group, but Emde (246) maintained that the methylamino group was equally susceptible of being shifted. Rabe (247) proved that the isomerism was not a question of position in a chain but of space about an asymmetric carbon atom. Emde (248), in his recent exhaustive examination of the steric phenomena of the ephedrine isomers, established their spatial structures.
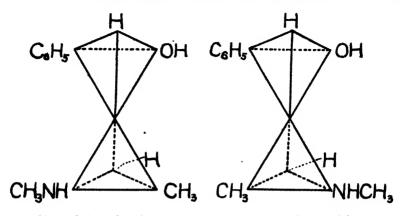
Designating the two asymmetric carbon atoms as "a" and "b," the optical possibilities become

$$
\begin{array}{cccc}
\mathrm{I} & \mathrm{II} & \mathrm{III} & \mathrm{IV} \\
\overbrace{} & & \overbrace{} & \\
\mathrm{a}+ & \mathrm{a}- & \mathrm{a}+ & \mathrm{a}- \\
| & | & | & | \\
\mathrm{b}+ & \mathrm{b}- & \mathrm{b}- & \mathrm{b}+
\end{array}
$$

and mixtures of the two pairs are then the racemic compounds. Arrangements I and II have the largest rotations, and these are therefore the dextro and the levo forms of pseudoephedrine (see table 5); III and IV depend on whether the rotation of "a" alone is greater or less than that of "b." From a study of phenyl-1-methylamino-2-propane or desoxyephedrine,

$$
\underset{\underset{\text{NHCH}_3}{|}}{\text{C}_6\text{H}_5\text{CH}_2\ \text{CHCH}_3}
$$

having for $[M]_D^{20}$ a value of $+33°$, Emde found the rotation of "a" greater than that for "b"; hence IV represents natural l-ephedrine and III represents the synthetic d-isomer.

From the fact that natural water-free ephedrine is an oil, which in the presence of water crystallizes as a monohydrate melting at 39–40°, and the fact that d-pseudoephedrine, melting at 118° does not, and probably cannot, take up water because of a betaine-like structure through the secondary alcoholic hydroxyl group, Emde believes that in the former the hydroxyl and methylamino groups the *trans* and in the d-pseudoephedrine they are *cis*. Therefore, are geometric configuration for the natural ephedrines may be given as follows:

Natural d-ψ-ephedrine   Natural l-ephedrine

and for the racemic forms

$$C_6H_5—CH—OH$$
$$CH_3—CH—NHCH_3$$

syn-form
Pseudoephedrine

$$C_6H_5—CH—OH$$
$$CH_3NH—CH—CH_3$$

anti-form
Ephedrine

The synthetic ephedrine is racemic and may contain mixtures of the two forms. Späth and Göhring (249) were the first to isolate the six isomers from a synthetic mixture.

Synthetic ephedrine is usually prepared by reducing catalytically the corresponding aminoketone

$$C_6H_5COCHCH_3$$
$$NHCH_3$$

In the commercial preparation of "ephetonin," nickel deposited on pumice or asbestos is used as catalyst. Catalytic reduction methods lead to the formation, predominantly if not purely, of racemic ephedrine (250, 251, 252), whereas reduction with sodium or sodium amalgam results in the formation largely, if not exclusively, of the pseudo ephedrine (253, 254, 255).

Physiologically, ephedrine is more active than pseudoephedrine. l-Ephedrine is more active than the d-ephedrine, while the racemic mixture possesses an activity intermediate between the two.

Kreitmar's (256) observation that the differences between *l*-ephedrine and *dl*-ephedrine (ephetonin) are very slight has not been confirmed by other investigators. Of the isomers of pseudoephedrine, the order of decreasing activity is from *d* to *dl* to *l*. It is interesting to note that here the levo isomer is less active than the dextro compound.

In view of the fact that the pharmacological properties of the various ephedrine isomers have been extensively reviewed and investigated by Chen, Wu and Henriksen (124) and Chen and Schmidt (93), it will suffice here to give in table 6 a summary based on the results of these authors.

TABLE 6

Pharmacological activity of ephedrine isomers

ISOMER	M.L.D. INTRAVENOUS TO RABBITS	PER CENT (AVERAGE) INCREASE IN BLOOD PRESSURE AFTER INJECTION OF 2 MG. INTO PITHED CAT WEIGHING ABOUT 2 5 KG.	RATIO OF ACTIVITY TO *l-ψ*-EPHEDRINE
l-ψ-	80	8	1:1
dl-ψ-	70	28	1:4
d-ψ-	75	37	1:6.8
d-	80	68.5	1:11.9
dl-	60	211	1:26.5
l-	60	280	1:35.1

In examining the effects of the oral administration of the various isomers in doses of 50 mg. to men, Chen, Wu and Henriksen found all to produce a rise in the systolic blood pressure, except *d*-ephedrine and *l*-pseudoephedrine. Since these two, in the light of the previous discussion, should be interconvertible by the mere "Umklappen" of the alcoholic hydroxyl, the question might logically be raised: Is such oral activity conditioned by the position in the molecule of the hydroxyl group?

That the spatial arrangement within the molecule has tremendous influence on its physiological activity is demonstrated by the ponderously abundant evidence. But the reasons for such wide and characteristic differences still remain to be determined.

XIV. SUMMARY

The general effect of the influence of structure on the pharmacological reactivity of epinephrine and similar compounds cannot be expressed in a few words. Perhaps a summary (see figure 2) with graphic formulas will serve much better. Since β-phenylethylamine possesses the minimum skeleton for optimum activity it becomes an excellent starting compound. The vectors indicate
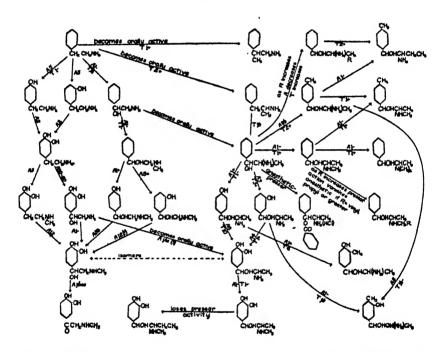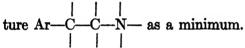

FIG. 2. INFLUENCE OF STRUCTURE ON THE PHARMACOLOGICAL REACTIVITY OF EPINEPHRINE AND SIMILAR COMPOUNDS

the derivatives obtained by single, successive substitutions. The number above the arrow, preceded by the letter "A" indicates the approximate change (when multiplied) in the pressor activity resulting from the substitution; in a similar manner the letter "T" followed by a number indicates the approximate change in toxicity.

Anyone who examines this summary, even though a casual observer, must be impressed with the sensitivity of the organism to what might be considered relatively minor modifications. Take, for instance, the effect of methyl substitution in phenylpropanolamine. There are indicated three such derivatives and what a difference there is in their respective physiological responses! If the chemist will consider, in addition, the mystifying difference between optical isomers, well may he envy the analytical capacity of living tissue. Whether the "protoplasmic reaction" is a resultant of physical forces or a consequence of chemical interplay has not been determined.

An examination of the approximations indicated in the summary will reveal certain general effects. Thus it is seen that:

1. Pressor activity is found in compounds possessing the structure Ar—C—C—N— as a minimum.

2. If the side chain is increased to three carbon atoms pressor activity is retained and oral activity is conferred. There is also a tendency for these compounds to produce their effect over a longer period.

3. If the side chain is increased beyond three carbon atoms the favorable action on the circulation is lost. The toxicity of the compound increases as the side chain becomes longer.

4. The alcoholic hydroxyl group in the side chain apparently serves to detoxicate the phenylalkanolamines, and in the catechol derivatives it also increases the pressor activity.

5. Modification of the amino group affects adversely the favorable action, serving to decrease the activity and increase the toxicity, these effects being roughly a function of the size of the substituted groups.

6. The aromatic portion of the molecule need not necessarily be a phenyl or a substituted phenyl group. Various naphthalene and heterocyclic derivatives also possess pressor activity.

7. Substitutions in the phenyl nucleus modify the circulatory effect, but apparently no substitution will—with a possible excep-

tion of simultaneous 2,4-dihydroxy substitution or substitution of a large alkyl group—completely eliminate this physiological action. The evidence thus far indicates that meta and para phenolic hydroxyl groups and a para chlorine atom increase the activity, while methyl groups influence the activity adversely.

However, not all of the conclusions are of equal validity; the reason for including those which are also doubtful is that the evidence now available indicates such trends. That there should be some legitimate doubt about some of these must necessarily follow from any attempt to compare and correlate the none too abundant data in a realm where there are so many unavoidable variables as there are in biological experimentation. Coupled with this, one not infrequently finds that an author generalizes on the basis of results which warrant only limited conclusions. Also at times the personality of the experimenter influences the interpretation of his results; he selects his result as supporting a conclusion drawn up in advance, when his complete results do not warrant such a procedure. Hence, for the present, a completely satisfactory and reliable correlation, much as it may be desired, is still impossible.

It is hoped not only that the future research on the pharmacodynamics of compounds such as these will yield more experimental data but that their significance may be properly interpreted. And it remains for the chemist, with the invaluable coöperation of his colleagues from the biological and physical sciences, to determine the operating mechanism which predetermines the nature or type of characteristic responses obtained from the respective reagents. Such studies appear especially worthwhile because of the added light they will throw on the general problem of the dependence of physiological behavior upon the structure of compounds. If this knowledge is ever to become available in its larger aspects it can be so only after the accumulation of more pertinent and reliable information. For it must not be forgotten that the advantage Mendeléeff enjoyed over Döbereiner and Newland lay perhaps not so much in his greater genius as in the additional knowledge at his disposal.

ACKNOWLEDGMENT

The author wishes to express his gratitude and appreciation to those who were ever ready with friendly criticisms and suggestions, particularly to his pharmacologist friends and to the editors, who have been of inestimable help in eliminating erroneous statements from the manuscript.

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